

Enantioseparation and structural optimisation of β -lactams and stilbene analogues for the treatment of triple negative breast and chemoresistant colorectal cancers



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Eavan C. McLoughlin, BSc. (Pharm), MPharm, MPSI, RSci.

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Under the supervision of

Asst. Prof. Niamh M. O'Boyle, BSc. (Pharm), PhD, MPSI

Co-supervised by Prof. Daniela M. Zisterer

Chapter 7 - Kinetic resolution of β -lactam combretazet racemates using *Candida antarctica* lipase B

7.1 Rationale for the use of lipase enzyme mediated kinetic resolution of β -lactams racemates

Our family of 3-hydroxyl analogues containing a variety of substitutions and deletions at the B ring *meta* position (Figure 7.1) have dual anti-cancer activity in both TNBC cells and chemoresistant HT-29 colorectal cancer cells.^{378,400} Chapters 3 and 4 focused on isolation of the corresponding enantiopure β -lactams through indirect chiral resolution. Chiral diastereomeric resolution has successfully resulted in enantioseparation of (+) and (–) 3-hydroxyl β -lactams as the *N*-Boc-L-proline derivatised 3*S*,4*S* and 3*R*,4*R* diastereomers using liquid chromatography (LC).⁷¹⁷ Using this method, enantiomers were isolated in very small quantities sufficient only for preliminary *in vitro* biological evaluation. An additional disadvantage noted was co-elution of diastereomers resulting in poorer *ee* of 50-80% for the second eluting 3*R*,4*R* diastereomer (Table 4.12). LC chiral resolutions were also labour and time intensive, requiring large volumes of organic solvent while yielding only minimal quantities of enantiopure β -lactams. O’Boyle *et al* have previously demonstrated that the aqueous solubility of β -lactam racemates is below the limit of detection measured using HPLC.³⁸⁰ Such solubility issues are potential limiting factors for pre-clinical progression of the eutomers described in Chapter 4 (Figure 7.1). Solubility issues for parent racemates have been overcome by derivatisation toward phosphate or amino acid prodrugs.³⁸⁰ Synthetic optimisation, solubility studies and biological evaluation of prodrug derivatives of lead enantiopure eutomers would require larger yields than chiral resolution has provided.

The biocatalytic approach involving kinetic resolution (KR) for the preparation of enantiomers was investigated, with the aim of increasing the isolated yields of enantiomers listed in Figure 7.1. This chapter explores the use of lipase enzymes, with focus on the use of CAL-B mediated methanolysis of 3-acetoxy β -lactam racemates (Figure 7.1). The use of KR holds potential as an improved strategy for isolation of larger yields of enantiopure 3-hydroxyl β -lactams in a greener and more sustainable manner for the purposes of progression towards the aqueous soluble prodrug derivatives. This chapter aimed to optimise an accessible biocatalytic method using

lipases for isolation of 3-hydroxyl enantiomeric β -lactams in comparable *ee*, but larger quantities, to chiral diastereomeric resolution. Preliminary phosphate prodrug derivatisation is explored with the aim of synthesising a biocompatible prodrug for future biological evaluation. Long term, it is our vision that a clinically viable prodrug will progress towards pre-clinical and clinical models as a novel agent for treatment of both TNBC and chemoresistant colorectal cancers.

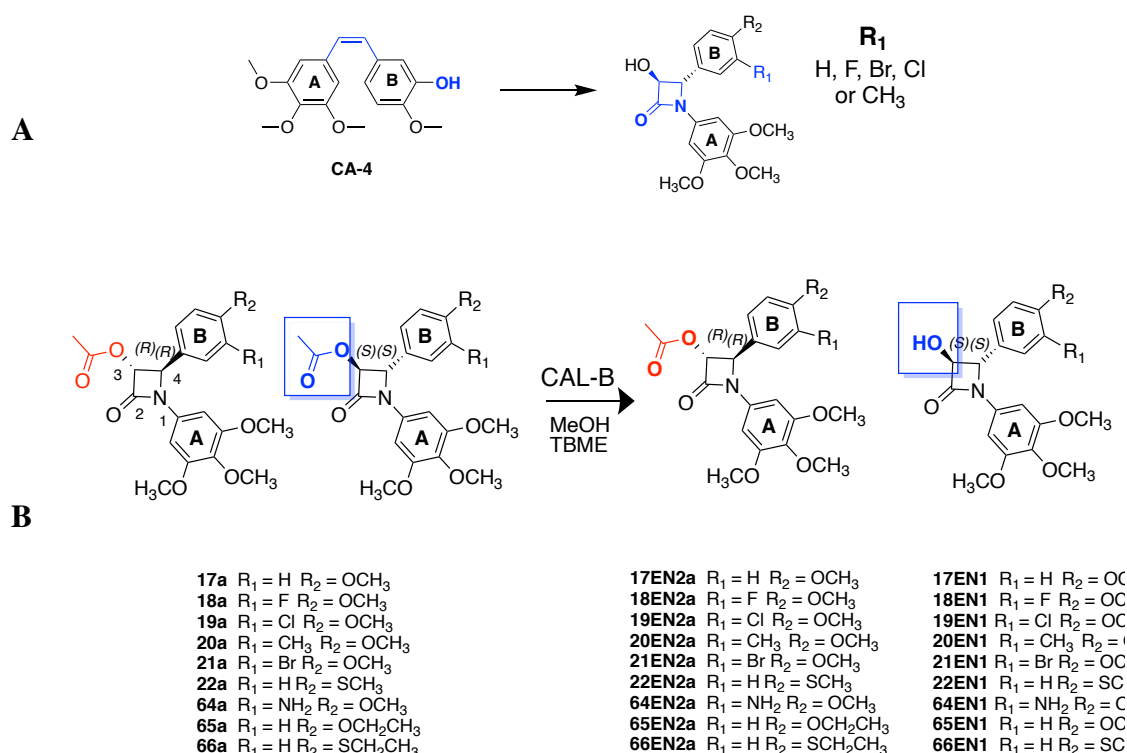


Figure 7.1: **A**: Structural modifications which prevent *cis/trans* isomerisation of CA-4 (β -lactam ring insertion) and increase anti-proliferative activity in CA-4 resistant HT-29 cells (replacement of the B ring *meta* hydroxyl moiety with a halogen or methyl group); **B**: Proposed CAL-B mediated methanolysis of the 3*S*,4*S* 3-acetoxy enantiomer to the 3*S*,4*S* 3-hydroxyl enantiomer.

7.2 Enantioselectivity values (*E* values) for KR reactions

To ensure high enantioselectivity, the rate of reaction for one enantiomer (k_{fast}) must be much greater than the rate of conversion for the second enantiomer (k_{slow}) (Figure 7.2). If the enantioselectivity is large, the rate of k_{slow} is negligible and the less reactive enantiomer remains unconverted by the enzyme. KR reactions would therefore cease

with excellent enantioselectivity at 50% conversion. In practice, this is rare. KR reactions often continue beyond 50% if the lipase enzyme of choice has poor enantioselectivity. Since the substrates are converted at different rates, their ratio in the reaction mixture is not constant as the reaction progresses, varying with degree of conversion. The enantiomeric ratio, E value, or selectivity factor, is a second order rate constant used to describe substrate specificity. The E value is determined by the environment of the system *i.e* substrate, enzyme, solvent, pH, temperature etc.⁷¹⁸⁻⁷²⁰ The E value is also dependent on the ee of substrate (ee_s), product (ee_p) and % conversion of reaction. E values are extremely useful constants for determination of KR enantioselectivities. Good E values are considered between 15-30 while above this value infers excellent enantioselectivity.⁷²¹

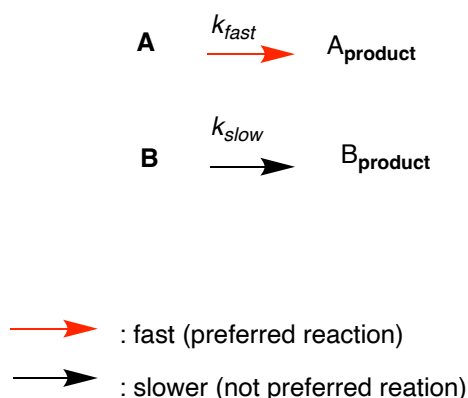


Figure 7.2: Biocatalytic kinetic resolution of racemates using enzymes

7.3 Lipase mediated KR of β -lactams and derivatives

There are several examples of lipase-mediated KR of β -lactams reported. For example, lipase B *Candida antarctica* (CAL-B) has been used for KR of β -lactam racemates for the production of optically pure β -amino acids and β -amino esters *via* enantioselective ring opening reactions, leaving behind a less reactive β -lactam.^{722, 723, 724} Lipases typically have the ability to form amide bonds but cannot cleave them due to their resonance stabilised carbonyl structures.⁷²⁵ A lack of resonance stabilisation for the β -lactam cyclic amides renders them susceptible to enantioselective ring-opening reactions by lipase enzymes. Ring opening reactions often require structural activation of the NH moiety of the β -lactam ring for *e.g* N -benzoyl protection to increase ring

reactivity.⁷²⁶ NH, *N*-acetyl and *N*-chloroacetyl racemates undergo enantioselective ring-opening reactions. In contrast *N*-*tert*-butoxycarbonyl and *N*-aryl substituted racemates have been determined as unsusceptible to methanolysis ring opening mediated by CAL-B.⁷²⁴ Steric factors may restrict this enzymatic ring opening reaction for *N*-aryl substituted β -lactams. Examples of lipase mediated enantioselective ring opening reactions of synthetic interest include the enantioselective ring opening reaction of *N*-unsubstituted [(3*R*, 4*S*)-(±)] *cis*-3-acetoxy-4-phenylazetidin-2-one using CAL-B in diisopropyl ether, for the preparation of the optically active taxane side chain component. CAL-B mediated a two-step cascade reaction involving cleavage of the acetoxy ester moiety to the free hydroxyl β -lactam, followed by a highly selective N1-C2 cleavage of the β -lactam ring resulting in *ee* of >98% for the desired β amino acid⁷²⁷ (Figure 7.3).

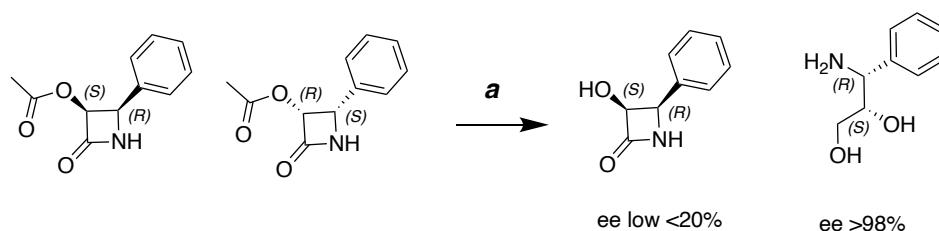


Figure 7.3: Enantioselective hydrolysis of 3-acetoxy *cis* β -lactam structure in a two-step cascade reaction to the enantioenriched 3-hydroxyl structure in <20% *ee* followed by enantioselective ring opening to enantiopure taxane side chain precursor in >98% *ee*.⁷²⁷ **a**: CAL-B, *i*Pr₂O, H₂O, 60 °C

KR has also been achieved for *N*-hydroxymethylated β -lactams *via* lipase catalysed hydroxyl *O*-acylation (**A**, Figure 7.4). *Burkholderia cepacia* (lipase PS) enantioselectively acylated the hydroxyl of the 4*S* enantiomer in presence of acyl donor vinyl butanoate in toluene containing 5% v/v acetone towards the esterified enantiomer, resulting in enantioseparation of racemic β -lactams in 98 and 80% *ee* (**A**, Figure 7.4). It was noted that cessation of the enzymatic resolution at 30% conversion was required in order to obtain high *ee* values for the preferentially hydrolysed *S* enantiomer, while continuation beyond 50% was necessary in order to obtain higher *ee* values for the

unreacted *R* enantiomer.⁷²⁸ This study also proposed a unique double resolution for the further enantioenrichment of the acylated *S* enantiomer beyond 80% *ee*. Lipase PS catalysed acylation using vinyl butanoate was quenched at 55% conversion (*via* filtration to remove the enzyme) followed by LC purification of *R* and *S* enantiomers in 98% and 80% *ee* respectively. The acylated *S* enantiomer derived from **A**, Figure 7.4 was subsequently subjected to lipase PS catalysed alcoholysis with 1-butanol, to yield >99% *ee*⁷²⁸ (**B**, Figure 7.4).

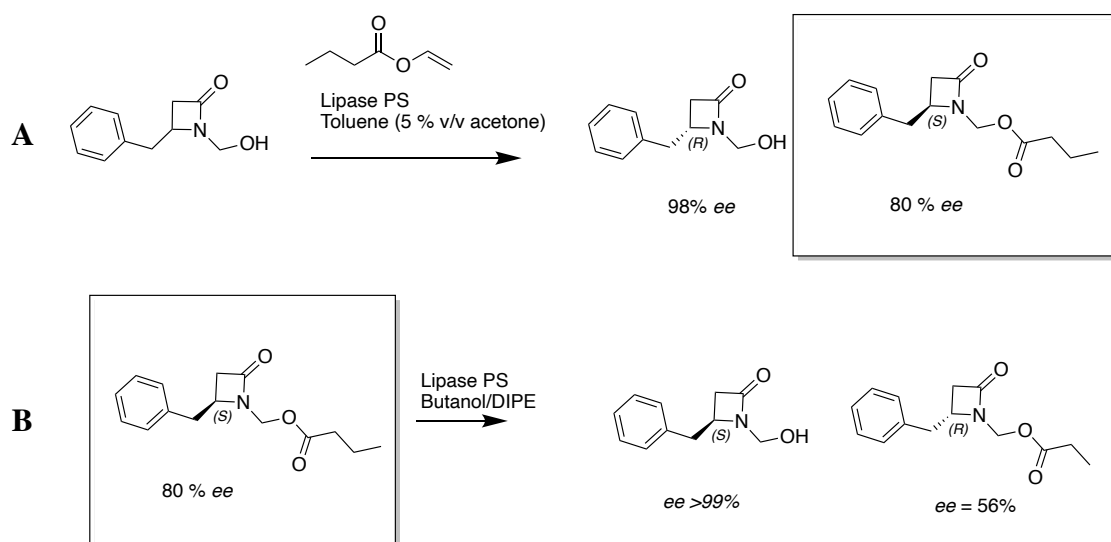


Figure 7.4: **A:** Lipase PS catalysed enantioselective acylation of β -lactams with vinyl butanoate;⁷²⁸ **B:** Lipase PS catalysed alcoholysis of the enantioenriched acylate (from **A**) as part of a double resolution strategy to yield the 4*S* enantiomer in >99% *ee*⁷²⁸

Carr *et al* have reported KR of 3-acetoxy β -lactam derivatives *via* hydrolysis in phosphate buffers (0.2 M pH 7.2) using lipase PS demonstrating that the enantioselective hydrolysis proceeded in favour of the 3*S* enantiomer. Conclusions were also made on the effects of the B ring substituent position, size and electronic effects on determining rate and enantioselectivity of reaction. *Para* electron withdrawing substitutions increased rate while electron donating substitutions had lower substrate conversions. *Ortho* electron withdrawing substitutions resulted in lower conversion rates. Conversion also increased with increasing size at the *para* position but decreased with increasing size at either *ortho* or *meta* positions.⁴¹⁹ These findings are of relevance for KR studies carried out in this chapter since 3-acetoxy β -lactams are

the substrates of choice. Additionally these 3-acetoxy substrates contain a variety of the substituents at both *para* and *meta* positions of the B ring (**B**, Figure 7.1).

7.4 Proposed mechanism for CAL-B mediated methanolysis of ester bonds

KR strategies reported in this thesis are based on methods reported by Sundell *et al* employing CAL-B and methanol for methanolysis.⁷²⁴ The CAL-B active site is a three dimensional structure exhibiting a canonical α/β fold, of parallel β sheets surrounded on both sides by α helices (Scheme 7.1).^{729, 730, 731} CAL-B's active site is tunnel shaped which sterically limits the position of bulky substrates.⁷³²⁻⁷³⁴ Its active site is composed of a catalytic triad: a serine (Ser), aspartic acid (Asp) and histidine (His) amino acid residue sequence (S105-H224-D187).^{732, 735} The Ser residue acts as the nucleophile in hydrolytic reactions, the His as an acid/base mediating catalytic transfer of protons between the enzyme and its substrate, while the Asp residue stabilises tetrahedral intermediates.⁷³⁶⁻⁷³⁸ The oxyanion hole contains hydrogen bond donors from amides of threonine (T40) and glutamine (Q106)^{739, 740} which stabilise the negative charge of tetrahedral intermediate oxygen atoms formed during catalysis.^{732, 741}

Lipases differ from typical esterases as they display two conformations: open or closed. In the closed conformation, a lid of amphiphilic α helices precludes the active site from reaching its substrate. Upon exposure of the lid to hydrophobic surfaces, lid displacement occurs, transitioning to the active site open form which in turn leads to interaction of enzyme with its substrate.⁷⁴² Most lipases exert their hydrolytic activity on the water-oil interface, a phenomenon known as interfacial activation. Interfacial activation is a two-step mechanism involving enzyme adsorption onto a hydrophobic interface followed by lid opening. Lid opening upon interfacial activation is therefore accompanied by a subsequent augmentation of lipolytic/enzymatic activity.⁷⁴³ In the absence of an aqueous-lipid interface, the lipase is rendered inactive, since exposure of the active site is energetically unfavourable, stabilising it in closed confirmation. CAL-B however is typically considered as an exception to the requirement for interfacial activation and does not require exposure to a hydrophobic surface to mediate its effects.⁷⁴⁴ The lid of CAL-B is composed of two amphiphilic α helices ($\alpha 5$ and $\alpha 10$). However it is now proposed that CAL-B's immobilisation on hydrophobic surfaces

mediates a form of interfacial activation and increases its activity towards bulkier substrates due to increasing the flexibility of the $\alpha 5$ helix at the lid of the active site which enables entry of a wider variety of substrates. CAL-B has a more open active site when immobilised on a hydrophobic surfaces, thus accommodating substrates that are not typically hydrolysed effectively, e.g. aromatic alcohols.⁷⁴⁵

The proposed serine hydrolase methanolysis mediated by CAL-B is illustrated as a two-step mechanism in Scheme 7.1. The first step of the catalytic cycle is an acylation reaction. The Ser side chain's oxygen acts as the nucleophile on the carbonyl of the ester substrate. This occurs concomitantly with proton transfer from the histidine residue to form the first tetrahedral acylated intermediate (INT₁). INT₁ is stabilised by the amino acid side chains of the 'oxyanion hole' (backbone amino and side chain hydroxyl of T40 and backbone amino of Q106). In the formation of this acyl intermediate, the 3-hydroxyl β -lactam is liberated (Scheme 7.1). The acyl intermediate is positively charged at the His224 residue, which is stabilised by Asp187.

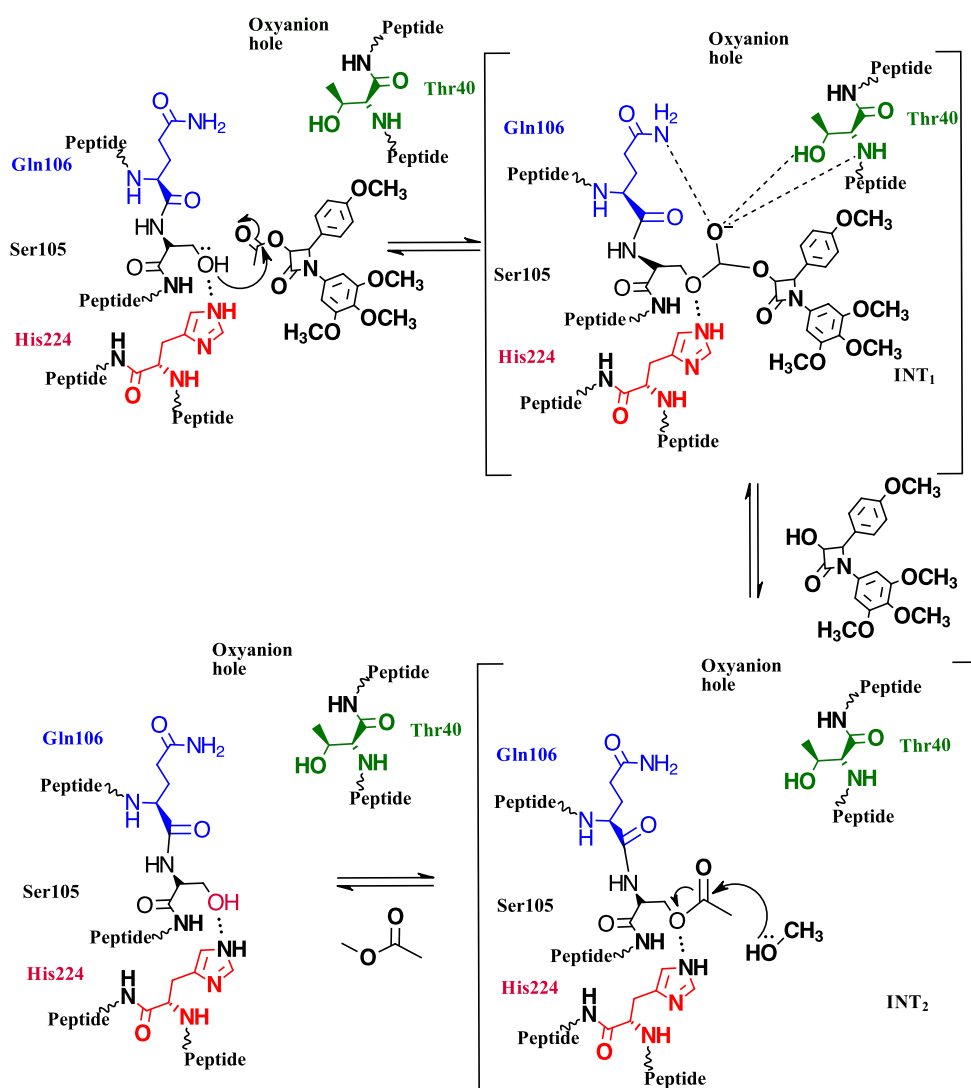
Secondly, a deacylation step occurs where a second tetrahedral intermediate (INT₂, Scheme 7.1) is formed by nucleophilic attack by the oxygen of an alcohol or water molecule. In the case of hydrolytic reactions, water acts as the nucleophile to form a second tetrahedral intermediate, which proceeds to regenerate the enzyme and release a carboxylic acid.⁷⁴⁶⁻⁷⁴⁸ In this thesis, CAL-B reactions are carried out in organic solvents, where methanol replaces water as the nucleophile.

CAL-B and other lipases preferentially hydrolyse water-insoluble substrates, demonstrating poor activity towards water-soluble substrates.⁷⁴⁹

This aims of this chapter are as follows.

- 1) Optimise a KR procedure for isolation of 3*S*,4*S* combretazets in similar or higher *ee* to diastereomeric chiral resolution.
- 2) Achieve a higher *ee* for the 3*R*,4*R* distomer compared to chiral diastereomeric resolution.
- 3) Exploration of prodrug synthesis.

- 4) Application of KR to the B ring *meta* hydroxyl substituted estomers derivatised with the *N*-Boc-L-proline CDR.



Scheme 7.1: The serine hydrolase mechanism mediated by CAL-B using methanol as a nucleophile. Oxyanion residues illustrated in blue. Acylated intermediate illustrated in red. INT₁ = tetrahedral intermediate 1. INT₂ = tetrahedral intermediate 2.

Since the combretazets are *N*-aryl substituted with the 3,4,5-trimethoxyphenyl A ring, this chapter aims to firstly explore the stability of the combretazet β -lactam scaffold to CAL-B and secondly potential reaction pathways of KR mediated by CAL-B.⁷²⁴

7.5 Preliminary kinetic resolution of 3-acetoxy racemate **17a** using CAL-B

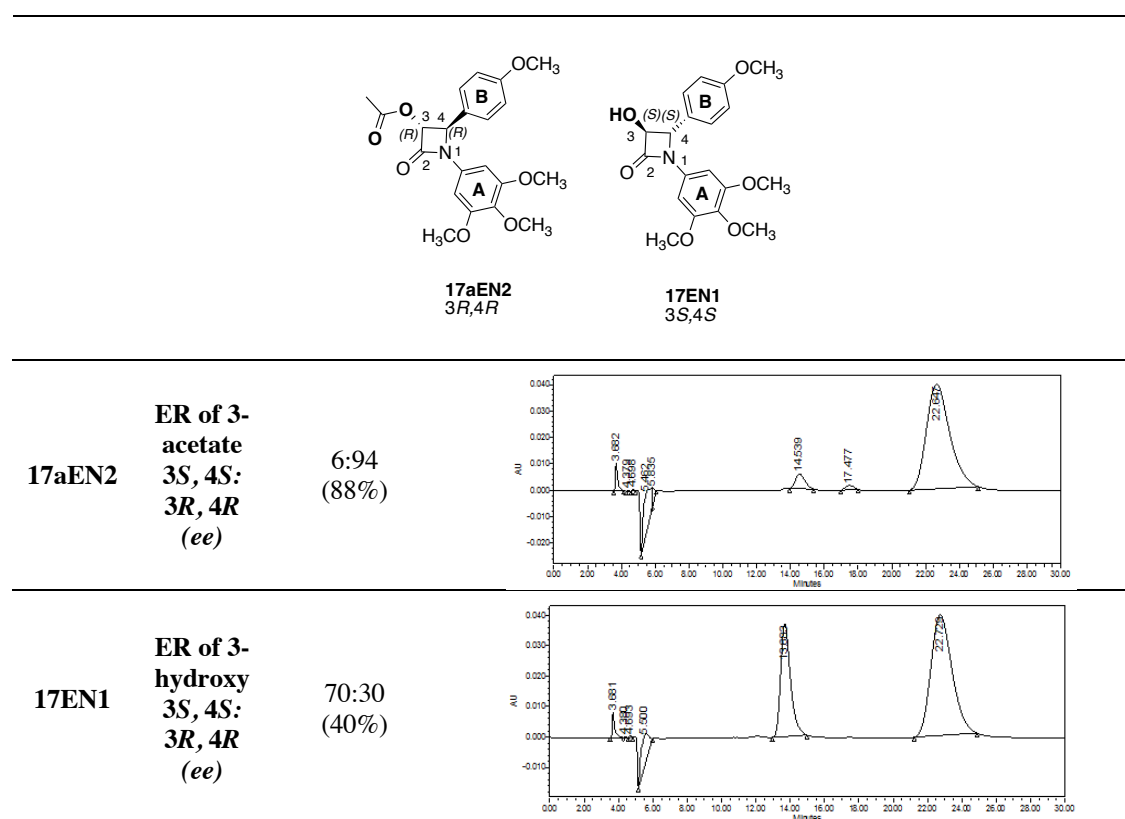
Optimisation of KR approaches were explored using racemate **17a**. Intermediate 3-acetoxy **17a** was subjected to alcoholysis (methanolysis) by selected lipase enzymes. Lipase B *Candida antarctica* (CAL-B) immobilised on Immunobead 150, recombinant from yeast 4022 U/g (Lot #BCCF8776, lot result 4022 U/g (Sigma) was selected for initial screening, based on a method reported by Sundell *et al.*⁷²⁴ Screening was also carried out with Amano Lipase PS, from *Burkholderia cepacia* (Lipase PS).

An initial quantity of 100 mg (402 U) of CAL-B of was chosen as a starting point with 100 mg (0.25 mmol) of **17a** (1mg/ mg of CAL-B/**17a**) in 10 mL of TMBE (Method A, Table 7.2). Preliminary screening of CAL-B aimed to determine if either enantioselective β -lactam ring opening or methanolysis of the 3-acetoxy moiety to an enantioenriched 3-hydroxyl product would proceed. TLC observations determined the formation of a new product and at 110 h the reaction was quenched by gravity filtration to remove CAL-B. Ring opening did not occur, as indicated by ¹H NMR analysis, but rather formation of the 3-hydroxyl product occurred in 50% yield (entry 1, Table 7.2). The β -lactam ring hydrogens at δ 5.37 ppm (H₃) and δ 4.85 ppm (H₄) for the 3-acetoxy enantioenriched substrate were accompanied by the presence of additional signals from the 3-hydroxyl H₃ and H₄ hydrogens at δ 4.79 ppm (H₄) and δ 4.74 ppm (H₃). These findings demonstrate the chemical stability of the β -lactam ring of **17a** in the presence of CAL-B towards ring opening alcoholysis and infers methanolysis of the 3-acetoxy toward the 3-hydroxyl moiety.

The methanol equivalents remained constant throughout each optimisation reaction while other parameters were selectively varied. A scale up was carried out for **17a** (1 mmol, entry 2, Table 7.2) in TBME (10 mL). After 48 hours only 7% conversion was observed. Chiral HPLC determined the *ee* of isolated substrates and products. Chiral HPLC employed an identical method as described in Chapter 4 using the chiral stationary phase column, Chrompak-IH-3 (150 x 4.6 mm), with a Chiral- IH-3 guard column and mobile phase of 1:1 *n*-hexane: isopropanol. In order to determine the absolute configuration for the preferentially hydrolysed enantiomer, the isolated 3-hydroxyl product (purified from unreacted 3-acetoxy substrate using LC) was

compared to standard enantiomers isolated in Chapter 3 using LC chiral resolution. In the case of the enantioenriched 3-hydroxyl **17**, the first eluting peak was assigned to the *3R,4R* **17EN2** by comparison to standard **17EN2** isolated in Chapter 3, while the second eluting and major peak was assigned to *3S,4S* **17EN1** (Table 7.1). The major peak for CAL-B methanolysed 3-hydroxyl products and second eluting enantiomer was therefore determined as the *3S, 4S* eutomer **17EN1** (as shown previously in Figure 4.2) while the enantioenriched and unreacted substrate was assigned to the *3R, 4R* 3-acetoxy distomer **17aEN2** (Table 7.1).

Table 7.1: Chiral chromatograms for enantioenriched *3R,4R* 3-acetoxy **17aEN2** and *3S,4S* 3-hydroxyl **17EN1** isolated from entry 6, Table 7.1 purified using LC post-reaction.



Entry 2 (Table 7.2) demonstrated poor *ee* (40%) for the 3-hydroxyl product. Entries 3-5 (Table 7.2) aimed to investigate whether scale or solvent volume determined the rate of reaction for CAL-B mediated methanolysis of 3-acetoxy **17a** to 3-hydroxyl **17**. Reactions were carried out at 0.25, 0.5 and 1 mmol scales (entry 3-5, Table 7.2)

increasing solvent volume to 10 mL of TBME per 0.25 mmol of **17a** (Method A, Table 7.2), to account for reaction scale. Using a lower scale (0.25 mmol), the methanolysis of 3-acetoxy to 3-hydroxyl eutomers proceeded faster with 30% and 47% conversion observed at 24h (1.25%/ h conversion) and 48h (0.75% /h) respectively. *Ee* for the 3-hydroxyl product remained modest determined as 32%. Increasing the scale to 0.5 and 1 mmol resulted in a slower rate of methanolysis and higher *ee* values. At 72h only 34% (compared to 39% on 0.5 mmol scale) of the 3-acetoxy **17a** had been converted to 3-hydroxyl **17** in 56% and 44% *ee*. Scale of the reaction influenced both reaction rate and *ee* values with greater conversion observed on the 0.5 mmol scale (47% by 48h) compared to 1 mmol scale (39% by 72h). Greater enantioenrichment was observed using the higher scale when a slower rate of CAL-B mediated was employed [32% versus 56% on 0.5 and 1 mmol scale respectively (entries 3 and 4, Table 7.2)]. Additionally, entries 4 and 5 (Table 7.2) gave rise to preliminary evidence that quenching the enzymatic reaction prior to 50% conversion may yield products in greater *ee*.

Entry 6 aimed to investigate reaction duration. CAL-B was combined with **17a** using identical conditions to entry 5 for 111 h. A higher rate of conversion was observed (69% versus 34%) and an excellent *ee* (88%) for the 3-acetoxy (3*R*,4*R*) enantioenriched substrate (**17a**). The *ee* value for the 3-hydroxyl product was however poor (40% versus 44% for entry 6 versus entry 5, Table 7.2). Enantioselectivity using Method A is poor for the desirable 3*S*,4*S* 3-hydroxyl eutomer. However the enantioenriched 3-acetoxy β -lactam was isolated in excellent *ee* of >85% when the reaction was allowed to proceed beyond 50% conversion.

Table 7.2: CAL-B optimisation reactions using **17a** as substrate.

Entry	Enzyme (mg)	Units	Scale mmol (mg)	Method	% conversion ^a	Time (h)	TBME (mL)	17aEN2	17EN1
								ER of 3-acetoxy 3 <i>S</i> , 4 <i>S</i> : 3 <i>R</i> , 4 <i>R</i> (<i>ee</i>)	ER of 3-hydroxyl 3 <i>S</i> , 4 <i>S</i> : 3 <i>R</i> , 4 <i>R</i> (<i>ee</i>)
1	100	402	0.25 (100)	A	50	110	10	Nd	Nd
2	400	1608	1 (400)	B	7	48	10	47:53 (6%)	70:30 (40%)
3	100	402	0.25 (100)	A	47	48	10	33:67 (34%)	66:34 (32%)
4	200	804	0.5 (200)	A	39	72	20	30:70 (40%)	78:22 (56%)
5	400	1608	1 (400)	A	34	72	40	39:61 (22%)	72:28 (44%)
6	400	1608	1 (400)	A	69	111	40	6:94 (88%)	70:30 (40%)
7	1200	5040	1 (400)	C	59	48	40	8:93 (85%)	70:30 (40%)
8	300	1206	1 (400)	D	35	144	10	37: 63 (26%)	63:37 (26%)
9	400	1608	1 (400)	E	51	192	100	22:78 (56%)	78:22 (56%)

^a = % conversion (alcoholysis from 3-acetoxy racemate to enantioenriched 3-hydroxyl β -lactam) measured using ¹H NMR in CDCl₃ at 400 MHz of reaction following work up)

ER = Enantiomeric Ratio, ***ee*** = enantiomeric excess (major – minor peak calculated using chiral HPLC)

10 equivalents of methanol used for all reactions

Method A: 1 mg CAL B / mg of **17a**. 10 mL of TBME / 0.25 mmol of **17a**, RT.

Method B: 1 mg CALB / mg of **17a**. 10 mL of TBME

Method C: 30 mg / mL of TBME, 10 mL of TBME / 0.25 mL of **17a**, RT

Method D: 30 mg / mL of TBME, solvent volume reduced to 10 mL, RT

Method E: 1mg/mg of CAL B/ mg of 17a, solvent volume increased 100 mL of TBME

To investigate the effect of increasing the rate of methanolysis, the enzyme content of CAL-B was increased to 30 mg/mL (Methods C and D, Table 7.2). 59% conversion was observed at 48h. *Ee* was poor (40%) for the 3-hydroxyl enantiomer but excellent (85%) for the 3-acetoxy enantiomer (entry 7, Table 7.2), due to >50% methanolysis. Reducing the solvent volume to 10 mL resulted in both a slower rate of and poor *ee* observed for both 3-acetoxy and 3-hydroxyl enantiomers (entry 8, Table 7.2). Since *ee* for the 3-hydroxyl enantiomer was low using 30 mg/ mL of CAL-B, 1 mg/ mL was trialed with a higher solvent volume (entry 9, Method E, Table 7.2). A combination of reducing the enzyme content and increasing volume yielded a more favourable *ee* of 56% for the desirable 3*S*,4*S* 3-hydroxyl enantiomer **17EN1**. The absolute configuration of the 3*S*,4*S* 3-hydroxyl enantiomer was confirmed using XRD analysis for entry 9 (Table 7.2) in 56% *ee* (Figure 7.5). XRD parameters are in agreement with those obtained for crystals isolated using chiral resolution (Table 4.7).

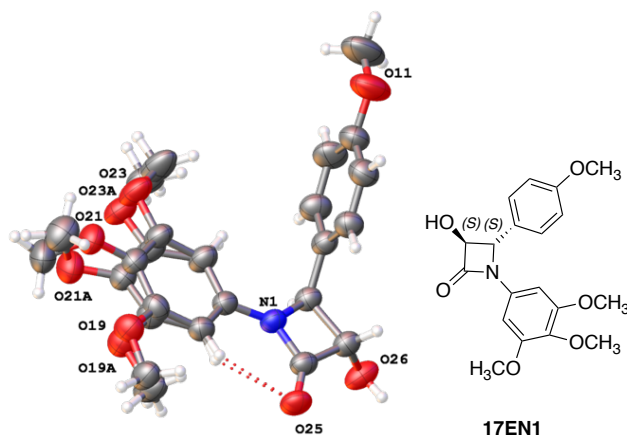


Figure 7.5: Disordered molecular structure of chiral 3-hydroxyl **17EN1** isolated by CAL-B mediated methanolysis in 56% *ee* (entry 9, Table 7.2). Atomic displacement shown at 50% probability and heteroatoms labelled only.

7.5.1 Time course analysis of enantiomeric excess for kinetic resolution of **17a**

While KR using CAL-B successfully yielded 3-acetoxy 3*R*,4*R* **17aEN2** in > 85 % *ee* (Table 7.1) which was superior to 71% obtained through chiral resolution (Chapter 4), further optimisation was required in order to develop a KR method for isolation of the 3*S*,4*S* 3-hydroxyl enantiomer in comparable *ee* values to chiral resolution as discussed in Chapters 3 and 4. A time course analysis of reaction 9 (Figure 7.6) was carried out in order to determine the optimal point of 3-acetoxy to 3-hydroxyl conversion yielding the

greatest *ee*, using chiral HPLC. However *ee* values were constant (56-62%) throughout the reaction (Figure 7.6).

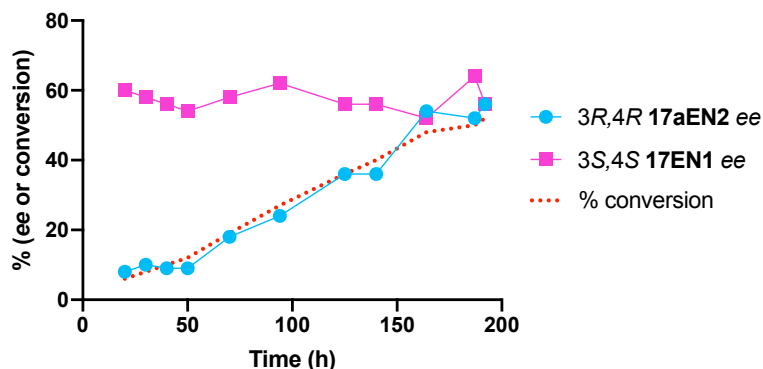
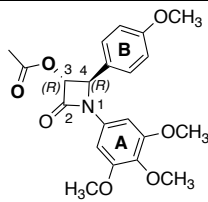
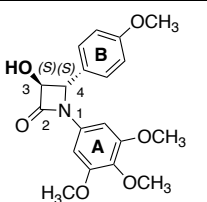


Figure 7.6: % *ee* of 3S,4S 3-hydroxyl **17EN1** and 3R,4R 3-acetoxy **17aEN2** versus % conversion for entry 9, Table 7.1

7.5.2 Enantioselectivity of CAL-B using methanol as solvent and **17a** as substrate

In order to investigate the effect of the methanol content on the enantioselectivity of CAL-B mediated methanolysis of 3-acetoxy racemate **17a** to the 3-hydroxyl 3S,4S enantiomer **17**, the reaction was carried out in methanol only. Approximately 95% conversion was observed within 3 hours. *Ee* was determined as 0% for both substrate and product (entry 1, Table 7.3). The reaction was repeated without enzyme in order to investigate solvent effects on conversion of the acetoxy to the free hydroxyl. In the absence of enzyme, conversion was not observed (entry 2, Table 7.3). These findings indicate that the rate of CAL-B mediated hydrolysis is greatly increased in the presence of methanol only and is not enantioselective. The 3-acetoxy is converted to the 3-hydroxyl racemate rather than an enantioenriched substrate.

Table 7.3: CAL-B optimisation reactions varying MeOH content using **17a** as substrate.

				17aEN2	17EN1	
17aEN2 3 <i>R</i> ,4 <i>R</i>		17EN1 3 <i>S</i> ,4 <i>S</i>				
Entry	Enzyme (mg)	Units	% conversion ¹	Time (h)	ER of 3-acetoxy 3 <i>S</i> , 4 <i>S</i> : 3 <i>R</i> , 4 <i>R</i> (<i>ee</i>)	ER of 3-hydroxyl 3 <i>S</i> , 4 <i>S</i> : 3 <i>R</i> , 4 <i>R</i> (<i>ee</i>)
1	400	1608	95%	3–4	1:1 (0%)	1:1 (0%)
2	0	0	0%	36	1:1 (0%)	Na

Reactions carried out on 1 mmol scale (400 mg of **17a**) in 40 mL of MeOH.

ER = enantiomeric ratio

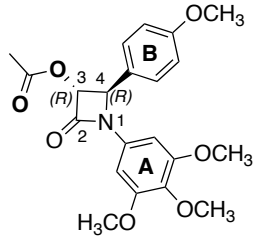
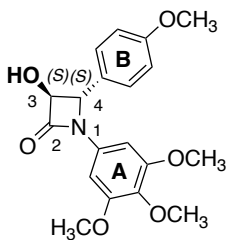
7.5.3 Investigating the nucleophilic effects of co-solvent and enzyme content on enantioselectivity of CAL-B with **17a** as substrate

Next the effects of reducing the methanol content to 2.5 eq rather than 10 eq (Table 7.2) on the *ee* was investigated. 11% conversion was observed after 3 days (70 h) with 74% of 3*S*,4*S* 3-hydroxyl **17** isolated (0.5 mmol scale, entry 1, Table 7.4). In contrast, the *ee* values were lower (62%) when the reaction scale was increased to 1 mmol (entry 3, Table 7.4). Conversion rates were poor (4% after 79h) using water as the co-solvent or nucleophile (entry 2, Table 7.4). Reducing the enzyme content to 0.5 mg/ mg of CAL-B per **17a** with 10 eq of methanol resulted in a larger *ee* (60%, entry 4, Table 7.4) than previously observed using 1 mg/ mg and 10 eq of methanol (40%, entry 5, Table 7.2). These findings indicate that a combination of the following may enhance *ee* for the 3*S*,4*S* 3-hydroxyl eutomer of **17**.

- 1) Reducing the methanol content to 2.5 eq.
- 2) Reducing the enzyme content by 50% to 0.5 mg/ mg of substrate.

Using a combination of reduced methanol and enzyme content, *ee* was increased to 62% for the 3-hydroxyl eutomer **17EN1** with 45% conversion after 78 h (entry 5, Table 7.4).

Table 7.4: Optimisation of CALB KR reactions for **17a** varying co-solvent, co-solvent equivalents and enzyme content

 17aEN2 3<i>R</i>,4<i>R</i>  17EN1 3<i>S</i>,4<i>S</i> 17aEN2 17EN1									
Entry	Enzyme mg (U)	Scale mmol (mg)	% conversion	Time (h)	TBME (mL)	Co-solvent	Co-solvent volume (eq)	ER of acetate 3 <i>S</i> , 4 <i>S</i> : 3 <i>R</i> , 4 <i>R</i> (<i>ee</i>)	ER of 3-hydroxyl 3 <i>S</i> , 4 <i>S</i> : 3 <i>R</i> , 4 <i>R</i> (<i>ee</i>)
1	200 (804)	0.5 (200)	11 %	70	20	Methanol	41 µL (2.5)	46:54 (8%)	13:87 (74%)
2	200 (804)	0.5 (200)	4%	79	20	Water	21 µL (2.5)	Nd	Nd
3	400(1608)	1 (400)	48%	72	40	Methanol	101µL (2.5)	75:25 (50%)	81:19 (62%)
4	200 (804)	1 (400)	35%	90	40	Methanol	404 µL (10)	34:66 (32%)	80:20 (60%)
5	200 (804)	1 (400)	45%	78	40	Methanol	101 µL (2.5)	75:25 (50%)	81:19 (62%)

7.6 Preliminary kinetic resolution of racemate **17** using amano lipase PS

KR using amano lipase PS from *Burkholderia cepacia* was trialed for the isolation of enantiomers of **17**. A 0.5 mmol scale reaction using 3-acetoxy **17a** and 1 mg/mg of lipase PS per **17a** at room temperature was carried out. Conversion to the 3-hydroxy β -lactam was not observed after 4 days using Method A adopted from Table 7.2 (entry 1, Table 7.5). The enzyme content was increased to > 5 mg / mg of lipase PS per **17a** and at 50 °C for 6 days, again with no observable conversion. Lipase PS was therefore pursued no further in this thesis.

Table 7.5: Preliminary KR reactions of **17a** using amano Lipase PS, from *Burkholderia cepacia*

Entry	Enzyme content (mg)	Enzyme content (U)	Scale mmol (mg)	Temperature	% Conversion	Time (h)
1	200	6000	0.5 (200)	RT	0	4 days (96)
2	300	10000	0.1 (40)	50 °C	0	6 days (144)

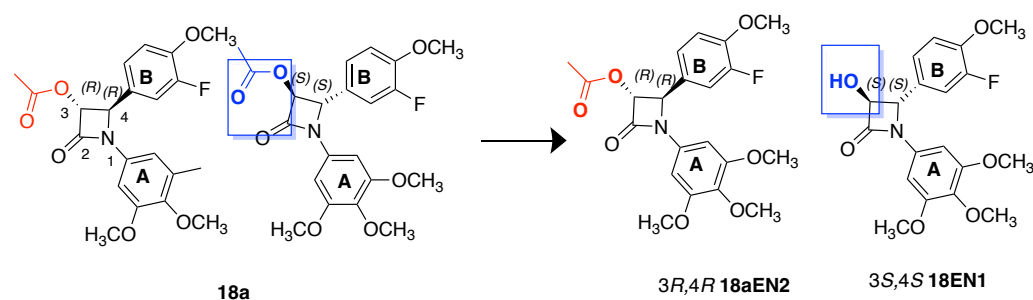
RT = room temperature

Reactions carried out in 20 mL of TBME with 10 eq of methanol

7.7 Preliminary kinetic resolution of racemate B ring meta substituted 3-acetoxy **18a** using CAL-B

Preliminary reactions demonstrated a much lower rate of methanolysis for the B ring meta fluorine substituted **18a** with respect to unsubstituted **17a** (entry 1, Table 7.6). After 64h only 7% conversion was observed. However *ee* was much greater (78%) than prior observations for **17a** (Table 7.2). Additionally increasing the volume from 40 mL (Method A, Table 7.6) to 100 mL (Method B, Table 7.6) resulted in a greater relative rate of conversion of 40% at 96 h (0.42%/h) versus 7% at 64h [0.11%/h, (entries 2 and 1, Table 7.6 respectively)] while maintaining a favourable *ee* of >75%.

Table 7.6: CAL-B optimisation reactions for substrate **18a** as substrate.



Entry	Enzyme content mg (U)	Scale mmol (mg)	Method	% conversion ^a	Time (h)	TBME (mL)	18aEN2	18EN1
							ER of 3-acetoxy 3S, 4S: 3R, 4R (<i>ee</i>)	ER of 3-hydroxyl 3S, 4S: 3R, 4R (<i>ee</i>)
1	419 (1685)	1 (419)	A	7%	64 h	40	73:27 (46%)	89:11 (78%)
2	419 (1685)	1 (419)	B	40%	96h	100	80:20 (60%)	88:12 (76%)
3	838 (838)	2 (838)	A	27%	168h	80	34:66 (32%)	78:22 (56%)

All reactions carried out with 10 eq of methanol

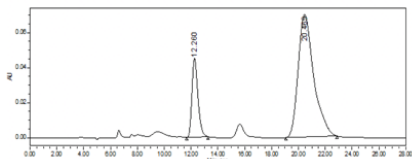
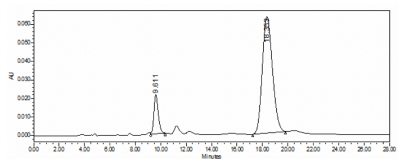
^a = % conversion (alcoholysis from 3-acetoxy to 3-hydroxyl measured using ¹H NMR in CDCl₃ at 400 MHz of reaction following work up)

ER = Enantiomeric Ratio, ***ee*** = enantiomeric excess (major – minor peak calculated using chiral HPLC)

Method A: 1 mg CAL-B / mg of 17a. 10 mL of TBME / 0.25 mmol of 17a, RT.

Method B: 1mg/mg of CAL-B/ mg of 17a, solvent volume increased 100 mL of TBME

Table 7.7: Chiral chromatograms for 3*R*,4*R* 3-acetoxy **18a** and 3*S*, 4*S* 3-hydroxyl **18**

ER of 3-acetate 3 <i>S</i> , 4 <i>S</i> 3 <i>R</i> , 4 <i>R</i> (<i>ee</i>)	3-acetoxy chiral chromatogram	ER of 3-hydroxyl 3 <i>S</i> , 4 <i>S</i> 3 <i>R</i> , 4 <i>R</i> (<i>ee</i>)	3-hydroxyl chiral chromatogram
80:20 (60%)		88:12 (76%)	

Chromatograms obtained from 3-acetoxy and 3-hydroxyl products isolated using LC post reaction from entry 2, Table 7.6.

7.7.1 Time course analysis of enantiomeric excess for CAL-B mediated KR of **18a**

A time course analysis of *ee* values during formation of the 3-hydroxyl 3*S*,4*S* eutomer was carried out in order to determine the time-point of greatest enantioselectivity. For entry 1, Table 7.6, *ee* values remained constant from 62-82% throughout the reaction illustrated as **A**, Figure 7.7. However for entry 2 (Table 7.6), *ee* values were highest (80-90%) at earlier time points of 25-35% methanolysis in comparison to 76% *ee* observed at 96 hours when 40% methanolysis had occurred (**B**, Figure 7.7). This data suggests that as KR approaches 50% methanolysis, the rate of non-selective mediated methanolysis of the 3*R*, 4*R* 3-acetoxy enantiomer increases. Therefore quenching the reaction prior to 40-50% conversion may increase the enantioselectivity of the reaction and yield greater *ee* for the desired 3*S*,4*S* eutomer. XRD analysis of **18a** confirmed the absolute configuration for 3-acetoxy **18a** and 3-hydroxyl **18** as 3*R*,4*R* and 3*S*,4*S* respectively. The XRD structure of **18a** whose absolute configuration was determined as 3*R*,4*R* is illustrated in Figure 7.8.

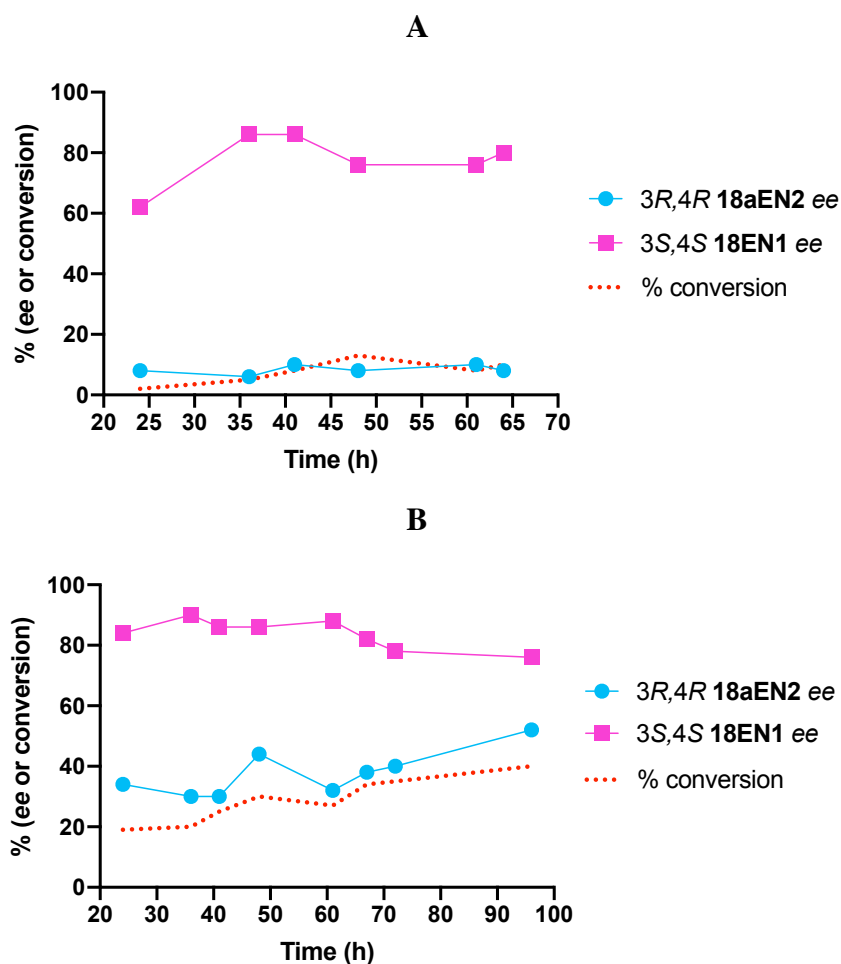


Figure 7.7: **A:** % *ee* for 3*S*, 4*S* **18EN1** and 3*R*,4*R* 3-acetoxy **18aEN2** (entry 1, Table 7.6) determined by chiral HPLC for **B:** entry 2, Table 7.6

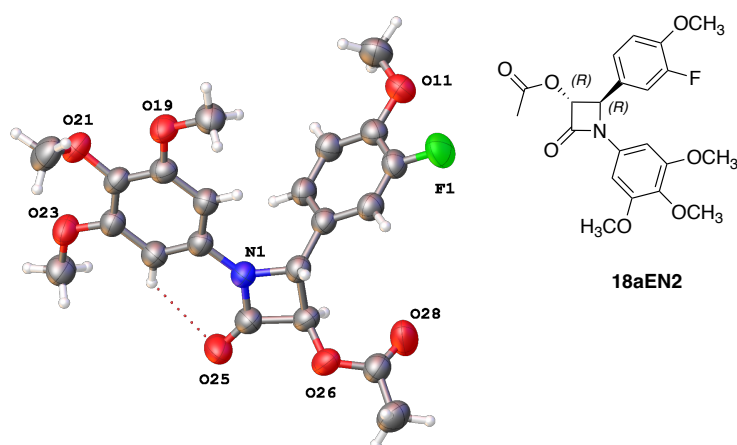


Figure 7.8: **A:** XRD structure of enantioenriched **18a** with absolute configuration 3*R*,4*R* isolated from CAL-B reactions, entry 2, Table 7.6, crystallised from methanol overnight.

A comparative illustration of the rate of CAL-B mediated methanolysis for **17a** versus **18a** is illustrated in Figure 7.9. In general, a greater rate of 3-acetoxy to 3-hydroxyl methanolysis was observed for B ring *meta* unsubstituted **17a** versus fluorine substituted **18a**. Since larger *ee* values were observed for **18EN1** (56-78%, Table 7.6) versus **17EN1** (30-56%, Table 7.2), a slower rate of methanolysis may be responsible for augmenting the enantioselectivity of KR reactions. The electron withdrawing *meta* fluorine substitution of **18a**'s B ring may favour this slower rate of reaction due to either electronic effects of modifying the conformation of the active site-ligand complex.

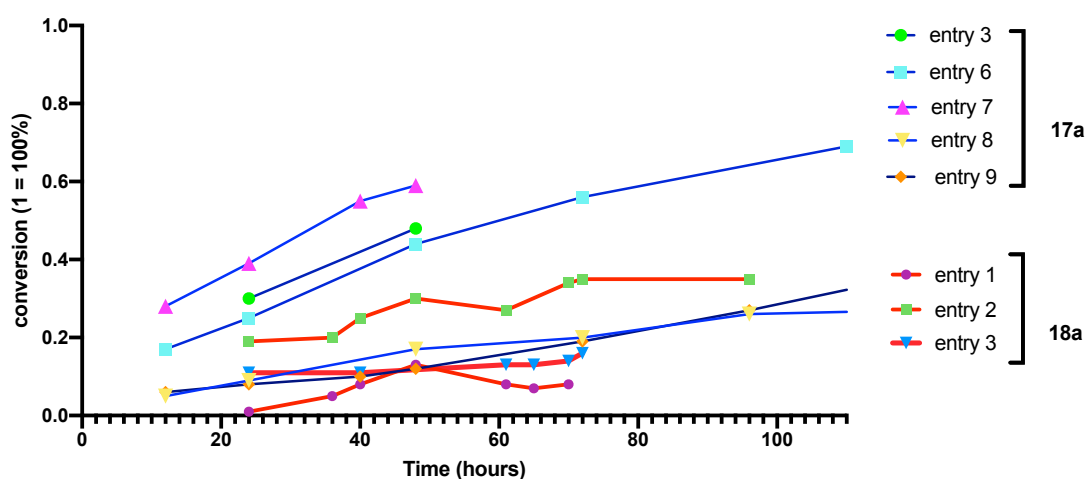


Figure 7.9: % conversion of **18a** (Table 7.6) and **17a** (Table 7.2) using CAL-B (determined by ^1H NMR in CDCl_3 at 400 MHz)

7.8 KR optimisation reactions for **20a** using CAL-B

Next CAL-B mediated KR was trialled with B ring *meta* methyl substituted **20a** (Table 7.8). Method A which employed 1 mg CAL-B / mg of **20a** and 10 mL of TBME / 0.25 mmol of **20a**, was trialled on a 0.5 mmol scale (entry 1, Table 7.8). These conditions in combination with **20a** as the substrate demonstrated rapid methanolysis by 72h accompanied by poor *ee* for 3*R*,4*R* 3-acetoxy **20a** (13%) and 3*S*,4*S* 3-hydroxyl **20EN1** (42%). Reducing the enzyme content to 0.5 mg/ mg of **20a** did not improve *ee* (34%, entry 3, Table 7.8). However, reducing both enzyme content and methanol equivalents (from 10 to 3 eq) yielded greater *ee* of 62% for 3-hydroxyl **20EN1**, quenching at at 50% conversion (entry 4, Table 7.8). Employing a combination of lower enzyme

content and methanol equivalents led to a much slower rate of reaction. For example, 50% conversion for **20a** was achieved by 144 h in contrast to 47% by 60 h for entry 2 (Table 7.8) when using a higher enzyme and methanol content.

Since favouring the slower rate of CAL-B mediated methanolysis appeared to increase the *ee* for 3*S*,4*S* 3-hydroxyl products, further optimisation would involve employing the lower enzyme content (0.5 mg/ mg of CAL-B per mg of 3-acetoxy racemate) in combination with 40 mL of TBME and a lower methanol content (3 eq of methanol), in addition to quenching of reactions at approximately 35% conversion. Quenching the reaction prior to 40-50% was anticipated to optimise *ee* in keeping with findings observed for **18a** in **B**, Figure 7.7.

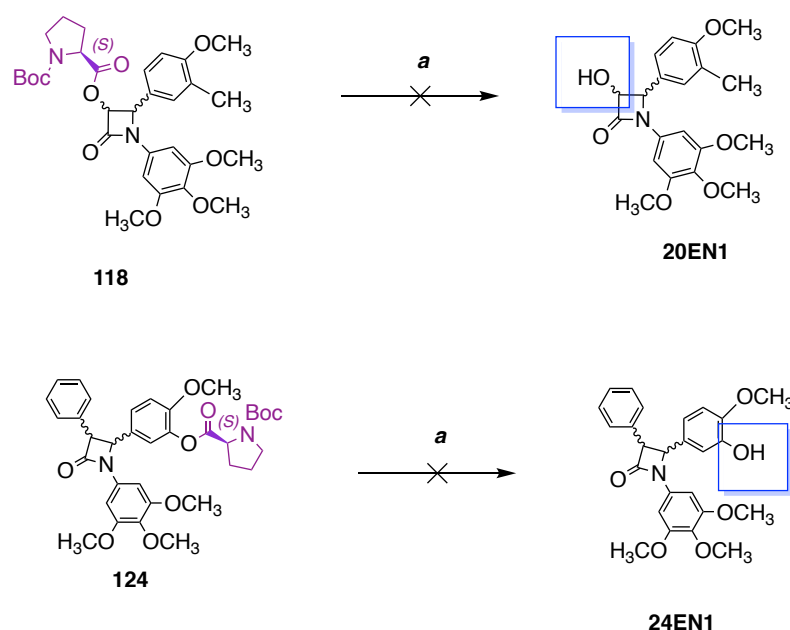
Table 7.8: CAL B optimisation reactions using **20a** as substrate.

								20aEN2 3 <i>R</i> ,4 <i>R</i>	20EN1 3 <i>R</i> ,4 <i>R</i>
								20aEN2	20EN1
Ent ry	Enzy me (mg)	Uni ts	Scale mmol	Meth od	% conversion ^a	Ti me (h)	Metha nol μL (Eq)	ER of 3- acetoxy 3 <i>S</i> , 4 <i>S</i> : 3 <i>R</i> , 4 <i>R</i> (<i>ee</i>)	ER of 3- hydroxyl 3 <i>S</i> , 4 <i>S</i> : 3 <i>R</i> , 4 <i>R</i> (<i>ee</i>)
1	207	843	0.5	A	31%	72	202 (10)	43:56 (13%)	29:71 (42%)
2	207	843	0.5	A	47%	60	202 (10)	68:32 (36%)	65:35 (30%)
3	103	222	0.5	B	57%	120	202 (10)	66:34 (32%)	67:33 (34%)
4	103	222	0.5	B	50%	144	62 (3)	72:28 (44%)	81:19 (62%)
Method A: 1 mg CAL B / mg of 20a . 10 mL of TBME / 0.25 mmol of 20a , RT. Method B: 0.5 mg/mg of CAL B/ mg of 20a , 10 mL of TBME / 0.25 mmol of 20a , RT									

7.9 Investigation of enantioselective CAL-B mediated deacetylation of *N*-Boc-*L*-proline derivatised diastereomers

Since a panel of *N*-Boc-*L*-proline derivatised diastereomers were already synthesised in Chapter 4 and CAL-B had demonstrated successful methanolysis of the 3-acetoxy ester bond, similar removal of the ester proline moiety from β -lactam racemates was investigated for both **118** and **123** as representative examples of B ring and 3-hydroxyl derivatised diastereomers (Scheme 7.2). After seven days, no methanolysis was observed on either ^1H NMR or TLC for either substrate. The steric bulk of the CDR in particular the presence of the *t*-butoxy moiety may prohibit entry of substrates **118** and **124** within the active site of CAL-B. Future work should focus on Boc removal to determine if decreasing the size of the acyl moiety would favour KR of proline diastereomers to furnish their desirable enantiomers.

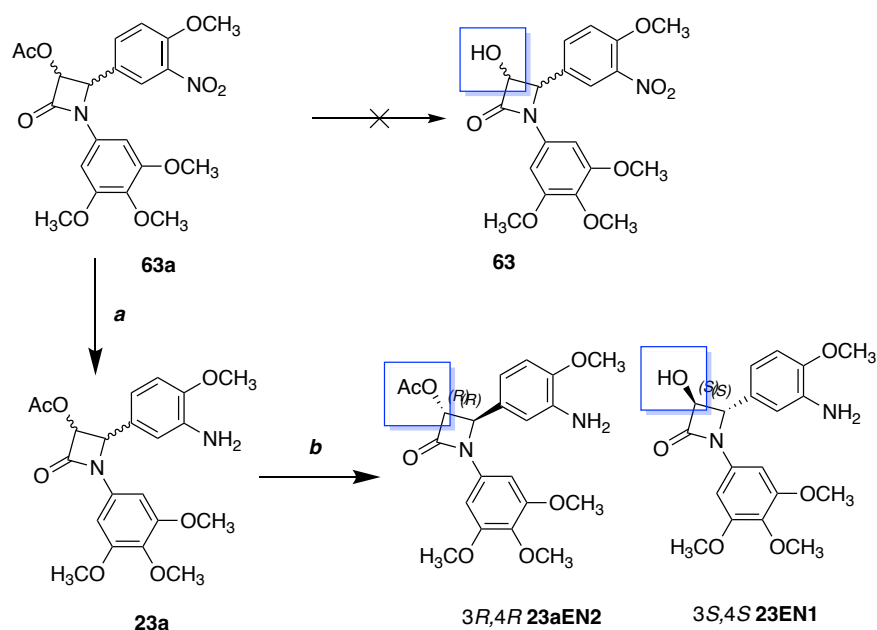
It is widely known that CAL-B preferentially hydrolyses fatty materials rather than water soluble substrates.⁷⁴⁵ Proline derivatisation may therefore also decrease CAL-B's preference for methanolysis of diastereomers **118** and **124** due to their increased water solubility with respect to the 3-acetoxy panel. To summarise steric hinderance and aqueous solubility of diastereomers synthetically designed for chiral resolution are factors which potentially prohibit CAL-B mediated KR. Future work could therefore focus on non-polar acyl derivatisation of the B ring *meta* hydroxyl of optimal size for entry into the CAL-B catalytic triad. Additionally, mechanochemistry is emerging as a strategy for expanding the substrate scope of CAL-B by modifying the shape of the lid $\alpha 5$ and $\alpha 10$ helices.⁷⁵⁰ Mechanical force may therefore enhance the enzyme's activity towards methanolysis of the B ring *meta* hydroxyl derivatised proline diastereomers.



Scheme 7.2: Proposed methanolysis of diastereomer mixtures synthesised for chiral resolution using. **a**: CAL-B 1 mg/mg scale of diastereomer mixture, MeOH, TBME

7.10 Preliminary KR of *trans* **63a** and **23a**

Enantiomers of amino-substituted β -lactam **23** were not isolated in Chapter 4. As previously mentioned, the *trans* racemate of either **23** or **63** are unreported to date in the literature. Since chiral resolution failed to isolate the corresponding 3*S*,4*S* eutomer **63EN1** and **23EN1**, KR was explored using 3-acetoxy **63a** as the substrate (Scheme 7.3). KR was not successful using 3-acetoxy **63a** but demonstrated success for the corresponding reduced racemate **23a**. The B ring *meta* amine substituted **23a** was successfully obtained by reduction of **63a** using zinc and glacial acetic acid over 6 days (a, Scheme 7.3). CAL-B methanolyse **23a** to the corresponding enantioenriched 3*S*,4*S* **23** in 50% *ee*. This data suggests that either the highly electron withdrawing effect or steric bulk of the nitro group prevents activity of CAL-B on substrate **63a**. In contrast by reducing the nitro to the corresponding amine, biocatalytic activity of CAL-B is observed to afford 3*S*, 4*S* **23EN1** in moderate *ee* (50%).



Scheme 7.3: Pathways for KR of **63a** and **23a** using CAL-B. **a**: Reduction of **63a** to **23a** using zinc and glacial acetic acid for 6 days. **b**: KR using CAL-B.

7.11 Investigation of lower enzyme and methanol content for KR of B ring meta substituted racemates

The use of 0.5 mg of CAL-B per mg of 3-acetoxy racemate in combination with 3 equivalents of methanol was further investigated to determine if the *ee* of the product could be ameliorated. Proposed substrates are detailed in B, Figure 7.1 and are specifically designed for dual anti-proliferative activity in both TNBC and HT-29 colorectal cancer cells. Optimisation was trialled for 3-acetoxy racemates **17a-21a** and afforded insight on the *ee* of 3-hydroxyl products obtained *via* CAL-B mediated methanolysis which depended on the B ring *meta* substitution. The *E* or enantioselective factor was calculated for each reaction (using the *ENANTIO* webtool⁷⁵¹) to comparatively illustrate CAL-B's enantioselectivity toward the 3*S*,4*S* enantiomer amongst the panel of 3-acetoxy β-lactam racemates.

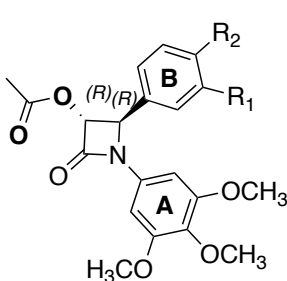
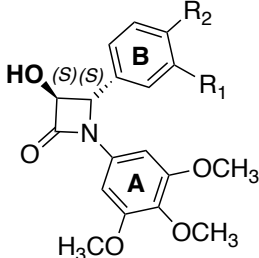
Ee for 3-hydroxyl enantiomers from reactions quenched at <50% (substrates **19a** and **21a**, Table 7.9) were much greater (70 and 83%) versus reactions which proceeded beyond 50%. For example **17a** had undergone 60% conversion at 4 days and yielded only 56% *ee* for 3-hydroxyl 3*S*,4*S* **17EN1**. However *ee* was determined as 91% for the 3-acetoxy 3*R*,4*R* enantiomer **17a**, when KR was allowed to proceed beyond 50%

conversion. This *ee* value was superior to the *ee* values obtained for the corresponding 3*R*,4*R* 3-hydroxyl **17EN2** *via* chiral resolution in Chapter 3.

This data suggests two important key findings

- 1) Quenching KR reactions at 35% conversion improves *ee* values for the desirable 3-hydroxyl 3*S*,4*S* enantiomer.
- 2) Allowing the reaction to proceed to 50% enables isolation of the 3*R*,4*R* 3-acetoxy diastomer in superior *ee* (*i.e.* > 91% *ee*) than has been observed for the 3*R*,4*R* 3-hydroxyl enantiomer obtained *via* chiral resolution.

Table 7.9: Optimisation of KR reactions for *meta* substituted 3-acetoxy β -lactams using CAL-B.

			
17aEN2	R ₁ = H R ₂ = OCH ₃	17EN1	R ₁ = H R ₂ = OCH ₃
18aEN2	R ₁ = F R ₂ = OCH ₃	18EN1	R ₁ = F R ₂ = OCH ₃
19aEN2	R ₁ = Cl R ₂ = OCH ₃	19EN1	R ₁ = Cl R ₂ = OCH ₃
20aEN2	R ₁ = CH ₃ R ₂ = OCH ₃	20EN1	R ₁ = CH ₃ R ₂ = OCH ₃
21aEN2	R ₁ = Br R ₂ = OCH ₃	21EN1	R ₁ = Br R ₂ = OCH ₃

Compound	Time	% conversion	3 <i>R</i> ,4 <i>R</i> 3-acetoxy		3 <i>S</i> ,4 <i>S</i> 3-hydroxyl		<i>E</i>
			Yield ¹ %	% <i>ee</i>	Yield ¹ %	% <i>ee</i>	
17a	4 days	60	27%	91	61%	56	11
18a	7 days	57	9%	82	41%	66	12
19a	6 days	35	18%	30	22%	70	8
20a	7 days	50	15%	72	16%	56	7
21a	5 days	30	61%	34	18%	83	15

Reaction conditions: All reactions carried out on 0.5 mmol scale, 0.5 mg of CAL-B per mg of 3-acetoxy β -lactam **17a-21a**, 50 mL of TBME, 3 eq of MeOH (62 μ L).

¹isolated yield for 3-acetoxy and 3-hydroxyl enantiomers respectively following LC.

The following optimised conditions for CAL-B mediated KR were selected based on these findings:

- 1) The pre-defined time point for quenching of reaction was ~35% conversion (30-50 considered as acceptable range ensuring to cease all reactions ideally prior to 50% conversion), measured using ^1H NMR in CDCl_3 at 400 MHz.
 - a. Pre-defined time point for **17a** and **22a** (*meta* unsubstituted analogues) was anticipated with a shorter endpoint.
- 2) Conditions employed were 3 eq of MeOH and 0.5 mg of CAL-B per mg of 3-acetoxy β -lactam substrate in 50 mL of TBME on the Radley carousel.
- 3) After the 3-acetoxy and 3-hydroxyl were isolated using LC, a second KR was carried out on the unreacted enantioenriched 3*R*,4*R* 3-acetoxy substrate to an end point of > 50% conversion.

7.12 Application of optimised KR for the isolation of 3*S*,4*S* 3-hydroxyl B ring *meta* substituted eutomers

The optimised method was applied to **17a** (Table 7.10). *Ee* values for **17a** ranged from 82-86% (average 84%) when the reaction was quenched from 35-41%, typically between 24-36 h. *E* values were between 16-24 (average 19). Higher *E* values were observed for reactions carried out on lower scales of 0.5 mmol (24) versus 1 mmol scale [**16** (Table 7.10)]. The validity of *E* values calculated using the *ENANTIO* online calculator were validated by comparing the measured % conversion *via* methanolysis versus the *ENANTIO* predicted % conversion. SEM values were within a range of acceptable experimental error. The *ee* of the enantioenriched 3-acetoxy 3*R*,4*R* β -lactams ranged from 44-60%, isolated in moderate yield of 24-58% for further reaction towards the enantiopure 3*R*,4*R* 3-acetoxy enantiomers (Table 7.10).

Excellent enantioselectivity was observed on a 0.25 mmol scale for **18a** (*E* = 28, *ee* of 86% for 3-hydroxyl **18EN1**) and 0.5 mmol scale (*E* = 14-16, *ee* of 83% for 3-hydroxyl **18EN1**). *Ee* values for the desirable 3*S*,4*S* 3-hydroxyl eutomer were excellent and constant ranging from 83-86%. The rate of conversion was slower for **18a** versus **17a** with only 15-36% conversion observed after 4-5 days for **18a** versus 30-40% conversion observed for **17a** between 24-48 h (Table 7.10). Solubility of **18a** in TBME was observed as poor, which may contribute to a slower reaction rate.

Table 7.10: Application of optimised KR procedure to 3-acetoxy β -lactam substrates **17a** -**23a** and **65**-**66a**.

Entry	Scale (mmol)	Time	conversion ¹	estimated conversion ²	SEM ³	3R,4R 3-acetoxy (s) yield	% ee _s	3S,4S 3-hydroxyl yield (p)	% ee _p	E ⁵
Substrate	17a				Product	3R,4R 17aEN2	Product	3S,4S 17EN1		
1	0.25	36 h	41%	39%	1.41	Nd	52	Nd	82	17
2	0.5	24 h	31%	39%	5.66	58%	54	30%	84	20
3	0.5	24 h	39%	41%	1.41	24%	60	6%	86	24
4	0.5	24 h	35%	39%	2.83	20%	54	17%	84	20
5	1	24 h	29%	35%	4.24	37%	44	36%	82	16
Substrate	18a				Product	3R,4R 18aEN2	Product	3S,4S 18EN1		
1	0.25	5 d	36%	47%	0.08	84%	76	6%	86	28
2	0.5	4 d	15%	22%	0.05	32%	24	16%	83	14
3	0.5	8 d	22%	17%	0.04	29%	18	11%	86	16
Substrate	19a				Product	3R,4R 19aEN2	Product	3S,4S 19EN1		
1	0.5	6 d	35%	36%	0.01	18%	40	22%	70	8
2	0.5	5 d	50%	36%	0.10	51%	44	28%	77	12
3	1	5 d	38%	34%	0.03	36%	42	17%	81	14
Substrate	20a				Product	3R,4R 20aEN2	Product	3S,4S 20EN1		
1	0.5	4 d	44%	44%	0	29%	56	21%	70	10
2	0.5	3 d	38%	39%	0.71	24%	64	17%	78	13
3	0.5	3 d	40%	42%	1.41	53%	52	24%	72	10
4	1	4 d	46%	40%	4.24	22%	56	24%	67	8
5	1	3 d	40%	43%	2.12	54%	52	26%	70	9
Substrate	21a				Product	3R,4R 21aEN2	Product	3S,4S 21EN1		
1	0.5	4 d	16%	18%	1.4	84%	18	25%	76	9
2	1	4 d	35%	38%	2.1	80%	42	30%	70	9
3	1	4 d	35%	36%	0.7	54%	40	23%	70	8
Substrate	22a				Product	3R,4R 22aEN2	Product	3S,4S 22EN1		
1	0.5	4 d	32%	34%	1.41	38%	42	42%	80	14
2	1	4 d	27%	29%	1.41	29%	32	17%	79	12
3	1	4 d	45%	47%	1.41	31%	66	34%	76	14
Substrate	23a				Product	3R,4R 23aEN2	Product	3S,4S 23EN1		
1	0.1	3 d	41%	39%	1.4	11%	40	8%	62	6
Substrate	65				Product	3R,4R 65aEN2	Product	3S,4S 65EN1		
1	1	6 d	22 %	24%		29%	24	4.3%	62	9
Substrate	66a				Product	3R,4R 66aEN2	Product	3S,4S 66EN1		
1	0.5	2 d	34%	44%	7.07	49%	64	21%	80	17
2	1	2 d	43%	49%	4.24	34%	66	25%	70	12

¹measured using ¹H NMR at 400 MHz in CDCl₃

² estimated % conversion (*calculated using ENANTIO online tool*)⁷⁵¹

³ SEM for actual conversions versus estimated conversion using *ENANTIO online tool*

⁴ Total 3-acetoxy (substrate) and 3-hydroxyl⁷⁵² yield

⁵ Enantiomeric ratio (*calculated using ENANTIO online tool*)⁷⁵¹

^s substrate ^p product

Excellent *ee* values of 70-81% were obtained for **19EN1**, the 3-hydroxyl 3*S*,4*S meta* chlorine substituted enantiomer when reactions were quenched between 35-50% conversion. On a 0.5 mmol scale, at 50% conversion an *ee* of 77% was determined demonstrating good enantioselectivity for the eutomer **19EN1** despite progression of KR beyond 35% (entry 2, Table 7.10). *Ee* values were slightly lower for 3*S*,4*S* **20EN1** (67-78%) and were typically greater when KR reactions were carried out on a 0.5 versus 1 mmol scale (Table 7.10). *E* values were quite poor (8-13). Nevertheless, the *ee* values for **20EN1** were determined as greater than *ee* values obtained *via* chiral diastereomeric resolution in Chapter 4 (Table 4.12) indicating the superiority of KR for resolution of **20**. Additionally the isolated quantity of 3-hydroxyl 3*S*, 4*S* **20EN1** (100 mg) in 70% *ee* (Table 7.10) was superior to the isolated yield obtained using chiral resolution.

KR for **21a** demonstrated moderate *ee* values of 70-76% with mild *E* values of 8-9. Nevertheless, KR using CAL-B yielded >100 mg of 3*S*,4*S* **21EN1** in 70% *ee* for future use in prodrug synthetic optimisation (Table 7.10). Excellent *ee* values and isolated yields were obtained for B ring 3-thiomethyl substituted 3*S*,4*S* 3-hydroxyl **22EN1** of 76-80% *ee* in quantities of 50-150 mg (Table 7.10). XRD analysis also confirmed the absolute configuration as 3*S*,4*S* for the 3-hydroxyl product **22EN1** isolated using KR (Figure 7.10)

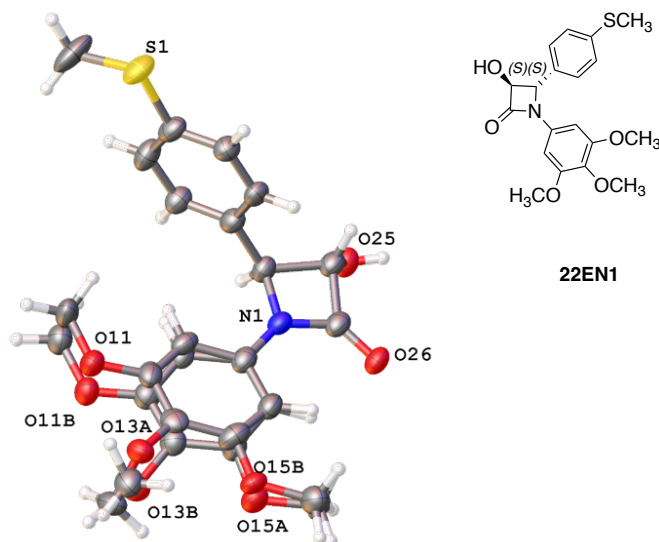


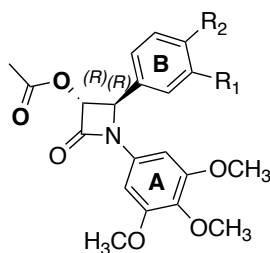
Figure 7.10: XRD for 3*S*,4*S* 3-hydroxyl **22EN1**. Disordered molecular structure with two locations for the trimethoxyphenyl ring (58:42%). Atomic displacement shown at 50% probability and heteroatoms labelled only.

The poorest *ee* values recorded using the optimised approach of 62% were for the desired 3*S*,4*S* enantiomer of **23EN1** (62%). The rate of methanolysis was fast for the *meta* amino substituted **23a** with 41% conversion observed by 3 days. This suggests that the strong electron donating effect of the amine substituent influenced the rate of CAL-B mediated methanolysis. By increasing the rate, the *ee* of 62% was lower than for example **18**, where only 36% conversion had occurred after 5 days in 86% *ee* (Table 7.10). The *E* value for **23** of 6 was the poorest of all *E* values recorded, indicating that CAL-B reacts with **23a** with poor enantioselectivity.

The *ee* value of 62% for B ring 4-ethoxy substituted **65a** was poorer in comparison to the corresponding 4-methoxy substituted analogue **17a** [average *ee* of 84%, (Table 7.10)]. In contrast the *ee* values for the 4-ethylthio substituted **66a** yielded excellent *ee* values of 70% and 80% and *E* values of 12 and 17 on 1 and 0.5 mmol scale respectively (Table 7.10). Isolated yields were good with approximately 50-100 mg recovered for prodrug optimisation.

7.13 Double KR for the isolation of 3*R*,4*R* 3-acetoxy diastomers in >90% *ee*

Enantioenriched 3*R*,4*R* 3-acetoxy β -lactams isolated from KR reactions in section 7.12, once purified using LC, were subjected again to a second CAL-B mediated KR otherwise referred to as a double resolution. The enzyme content was increased to 1 mg of CAL-B per mg of 3-acetoxy substrate while 6 eq of MeOH was employed. Despite increasing both the enzyme content and the methanol equivalents, the rate of reactions were slower. For example, only 52% conversion was observed for **17a** (commencing at 37% *ee*) after 4 days, which was determined as 30-39% conversion for the parent racemate (0% *ee*) at only 24 hours (Table 7.11). This suggests that the activity of CAL-B towards the 3*R*,4*R* enantiomer is less favoured and confirmed that while KR reactions are not 100% enantioselective, the 3*R*,4*R* enantiomer is not optimally methanolysed by CAL-B. Excellent *ee* values were obtained for the 3*R*,4*R* 3-acetoxy enantiomers of 96-99% *ee*, by allowing the reaction to approach or progress beyond 50%. As anticipated the *ee* values for the 3-hydroxyl enantiomers using the double resolution strategy were extremely poor [20-56% *ee*, (Table 7.11)].

Table 7.11: Isolation of 3-acetoxy 3*R*,4*R* distomers using CAL-B

17aEN2 R₁ = H R₂ = OCH₃
18aEN2 R₁ = F R₂ = OCH₃
19aEN2 R₁ = Cl R₂ = OCH₃
20aEN2 R₁ = CH₃ R₂ = OCH₃
21aEN2 R₁ = Br R₂ = OCH₃
66aEN2 R₁ = H R₂ = SCH₂CH₃

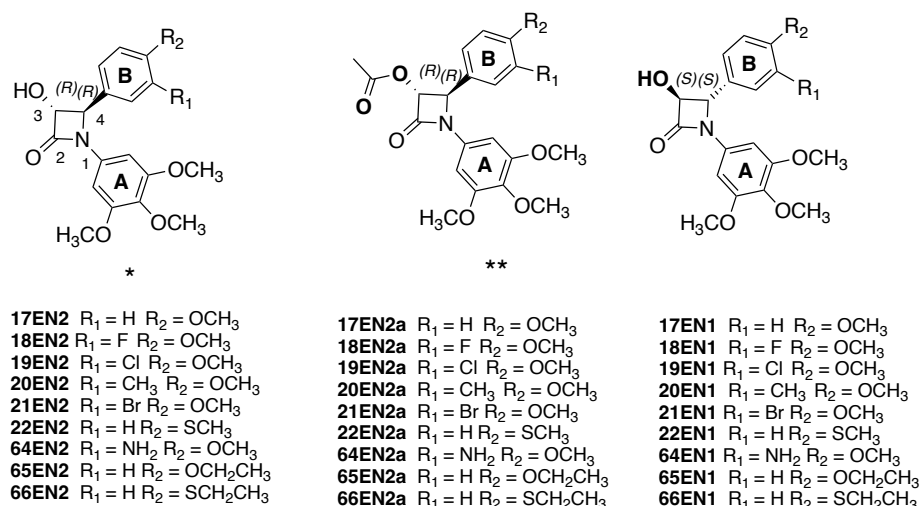
Compound	% <i>ee</i> prior to reaction	Scale (mmol)	Time	conversion	3- acetoxy (s) yield	% <i>ee</i> _s	3- hydroxy yield (p)	% <i>ee</i> _p	<i>E</i> value
17aEN2	37	4 d	52%	68%	30%	99	56	46	13
19aEN2	51	5 d	43%	75%	36%	99	20	33	9
21aEN2	40	3.5 d	63%	99%	16%	96	10	10	2
22aEN2	66	3.5 d	42%	80%	20%	98	20	24	7
66aEN2	64	5 d	36%	81%	16%	99	34	34	9

Reactions carried out at 1 mg/ mg of CAL-B per mg of 3-acetoxy β-lactam and 6 eq of MeOH

7.14 Comparison of *ee* values obtained via chiral diastereomeric resolution versus kinetic enzymatic resolution

Table 7.12 illustrates comparative *ee* values obtained using two independent methods for enantioseparation of β-lactam racemates; chiral diastereomeric resolution versus kinetic enzymatic resolution. Values for the 3*S*,4*S* enantiomers are comparable and in some cases KR was superior for enantioseparation *e.g* for **18EN1** KR resulted in 86% *ee* versus 78% *ee* isolated *via* chiral diastereomeric resolution. For the 3*R*,4*R* enantiomers KR using the two step optimised procedure yielded enantiopure 3-acetoxy enantiomers in greater *ee* to the corresponding 3*R*,4*R* 3-hydroxyl enantiomers isolated *via* chiral diastereomeric resolution. As discussed previously, due to co-elution of diastereomers during LC chiral resolution, poor *ee* values were continuously observed for the 3*R*,4*R* enantiomer. For example, chiral resolution yielded **17EN2** is 71% *ee* versus KR which yielded the corresponding 3-acetoxy in 99% *ee*.

Table 7.12: Comparison of *ee* values obtained using *N*-Boc-L-Proline as the CDR in diastereomeric resolution versus kinetic resolution with lipase enzyme CAL-B.



* Isolated only via diastereomeric resolution

** Isolated only via KR

Compound	<i>Ee</i> (%) obtained via diastereomeric resolution	Compound	<i>Ee</i> (%) obtained via kinetic resolution
17EN1 (S,S)	94	17EN1 (S,S)	74
17EN2 (R,R)	71	17aEN2 (R,R)	99
18EN1 (S,S)	78	18EN1 (S,S)	86
18EN2 (R,R)	75	18aEN2 (R,R)	60 ¹
19EN1 (S,S)	91	19EN1 (S,S)	81
19EN2 (R,R)	78	19aEN2 (R,R)	99
20EN1 (S,S)	66	20EN1 (S,S)	78
20EN2 (R,R)	50	20aEN2 (R,R)	68
21EN1 (S,S)	85	21EN1 (S,S)	76
21EN2 (R,R)	84	21aEN2 (R,R)	96
22EN1 (S,S)	84	22EN1 (S,S)	81
22EN2 (R,R)	85	22aEN2 (R,R)	98
23EN1 (S,S)	Not isolated ²	23EN1 (S,S)	61
23EN2 (R,R)	Not isolated ²	23aEN2 (R,R)	Nd
65EN1 (S,S)	Not isolated ³	65EN1 (S,S)	76
65EN2 (R,R)	Not isolated ³	65aEN2 (R,R)	Nd
66EN1 (S,S)	Not isolated ³	66EN1 (S,S)	80
66EN2 (R,R)	Not isolated ³	66aEN2 (R,R)	99

Method with greatest *ee* highlighted in bold writing.

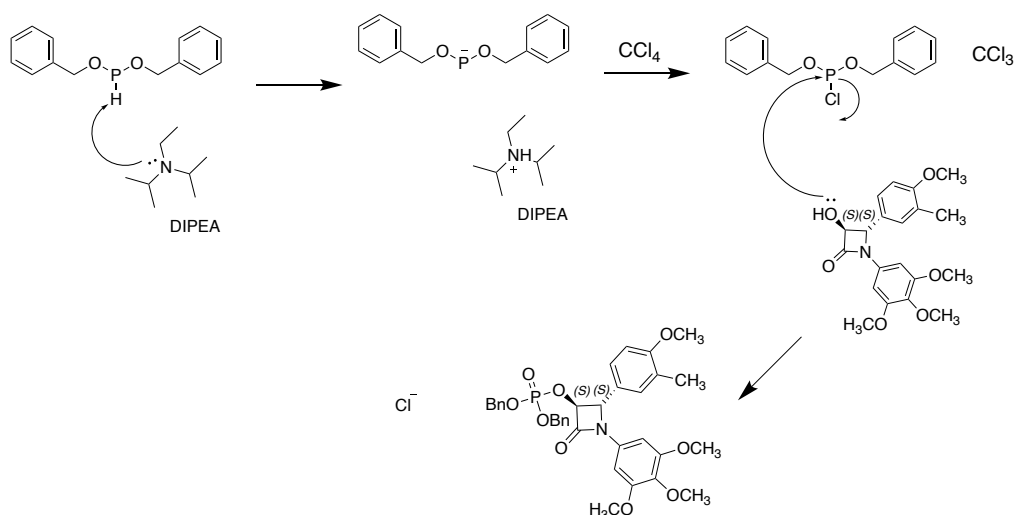
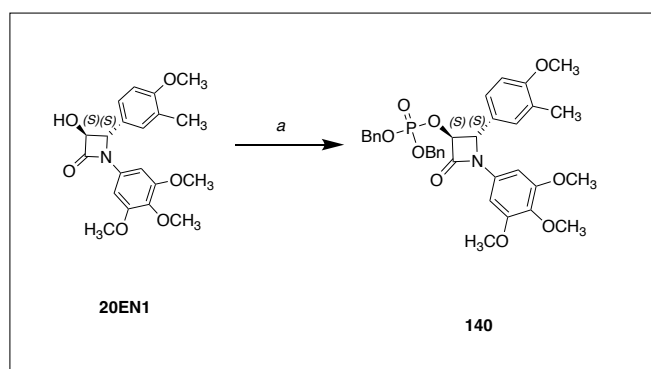
¹not isolated with optimised CALB kinetic resolution procedure.

²chiral diastereomeric resolution unsuccessful for isolation of enantiomers.

³chiral diastereomeric resolution not carried out.

7.15 Preliminary phosphate prodrug synthesis

Phosphate prodrug synthesis was investigated for 3*S*,4*S* 3-hydroxyl enantiomer **20EN1** which has been isolated in 70% *ee* using KR (Table 7.10). The chemistry for phosphate ester derivatisation involved use of carbon tetrachloride (CCl₄), DMAP, dibenzyl phosphite and DIPEA to form the dibenzyl phosphate ester of eutomer **20EN1** and has been previously described by our group for phosphate ester derivatisation of racemate **24** (Scheme 7.4).³⁸⁰



Scheme 7.4: Synthesis of **140** as the dibenzyl phosphate ester of **20EN1** via Atherton-Todd reaction.⁷⁵³ **a**: CCl₄ (1.4 eq), ice at 0 °C for 15 minutes in an inert atmosphere, MeCN. Then DMAP, DIPEA and dibenzylphosphite at 0 °C. Then RT overnight.

^1H NMR at 600 MHz in CDCl_3 confirmed successful coupling of dibenzyl phosphite to **20EN1** to yield **140** in 50% yield. The purified ^1H NMR demonstrates a multiplet at δ 5.10 ppm which corresponds to four benzyl protons. The phosphorous is observed on the ^{31}P NMR spectrum at δ -2.84 ppm (**B**, Figure 7.11). The phosphorous of dibenzyl phosphate couples to H_3 of the β -lactam ring to form a double doublet with a $^3J_{\text{P-H}}$ coupling constant of 7.5 Hz, while a ^3J coupling constant to H_4 is measured as 1.8 Hz (**A**, Figure 7.11). PYSCHÉ (pure shift yielded by chirp excitation) NMR experiments are broadband ^1H decoupled ^1H NMR spectra and remove proton J coupling⁷⁵⁴⁻⁷⁵⁶ enabling visualisation of the ^{31}P coupling for H_3 to the dibenzyl phosphate moiety. A doublet with a J value of 8.5 Hz is observed on the PYSCHÉ spectrum at δ 5.06 ppm while H_4 resonates as a singlet at 4.94 ppm due to proton decoupling (blue, **C**, Figure 7.11) rather than the doublet with a ^3J value of 1.8 Hz observed on the ^1H NMR spectrum (red, **C**, Figure 7.11). This data supports the hypothesis that synthesis of phosphate ester prodrugs of the 3-hydroxy panel of combretazet 3*S*,4*S* enantiomers is synthetically accessible. Future work will involve application and further optimisation of this chemistry to yield a panel of phosphate prodrugs for further physiochemical and biochemical analysis

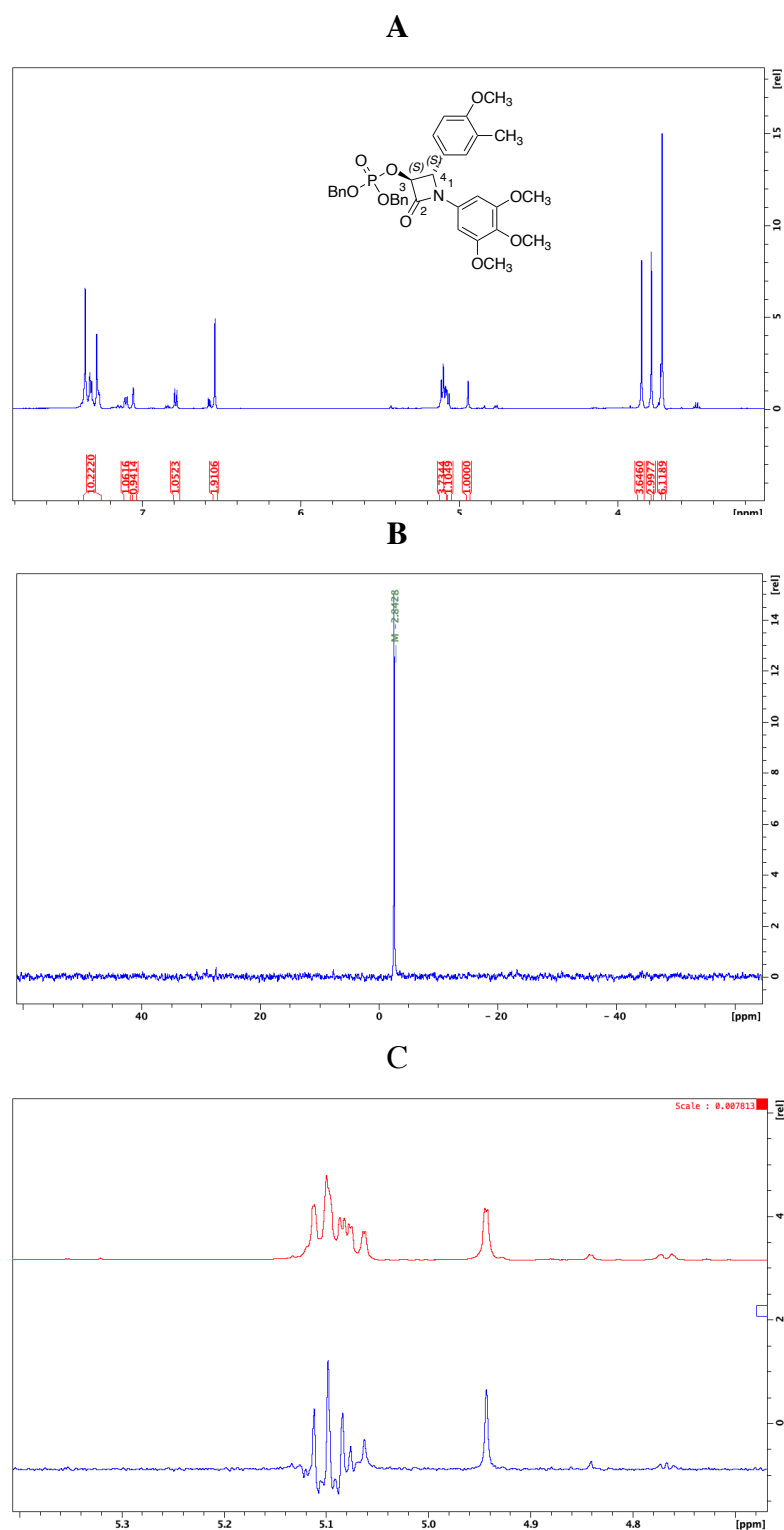


Figure 7.11: NMR spectra for dibenzyl phosphate derivative of *3S,4S* **140** (70% *ee*) **A**: ^1H NMR in CDCl_3 at 600 MHz. **B**: ^{31}P NMR spectra at 162 MHz **C**: Blue: PYSCHÉ for **140** (dibenzyl phosphate ether of **20EN1**) at 600 MHz in CDCl_3 in H_3 and H_4 region illustrating the $^3J_{\text{P-H}}$ coupling at δ 5.05 ppm with of 8.5 Hz while H_4 resonates as a singlet. Red: ^1H NMR spectrum for H_3 and H_4 at 600 MHz in CDCl_3 .

7.16 Conclusions and future outlook

In summary, in this chapter enantioseparation of a panel of β -lactam enantiomers has been achieved using a biocatalytic approach with the lipase enzyme CAL-B. Preliminary data illustrated that CAL-B did not mediate ring opening reactions for 3-acetoxy β -lactams but instead renders the 3-acetoxy structures susceptible to CAL-B mediated methanolysis towards the 3-hydroxyl enantioenriched eutomers. Initial results demonstrated poor enantioselectivity and moderate enantioenrichment only. Reducing the enzyme content to 0.5 mg/mg with 3 equivalents of methanol in TMBE greatly enhanced the *ee* (70-80%). XRD analysis confirmed the 3-hydroxyl product as the desirable 3*S*,4*S* eutomer. Additionally the 3*R*,4*R* distomer was isolated in >95% *ee*. This provides strong rationale to support KR as a promising strategy for resolution of a panel of racemic 3-hydroxyl β -lactams in high *ee* comparable to chiral diastereomeric resolution. KR is a rapid, accessible and sustainable strategy for enantioseparation, removing the need for expertise in LC purification and use of large volumes of organic solvents. KR is advantageous for enabling rapid access to large yields of β -lactam enantiomers for synthetic exploration of the most biocompatible prodrug, from a range of phosphate and amino acids, for progression towards pre-clinical and clinical models as a novel agent for treatment of both TNBC and chemoresistant colorectal cancers.

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Chapter 8 -Physiochemical analysis, prodrug synthesis and structural optimisation of Pyrazinib

8.1 Pyrazinib (**P3**) as a promising radiosensitiser

Pyrazinib [(*E*)-2-(2-(pyrazin-2-yl)vinyl)phenol or **P3** has demonstrated promising anti-angiogenic and radiosensitising activity both *in-vitro* and *in-vivo* as discussed in the introduction to this thesis.^{448, 449, 451} Its physiochemical properties have not been evaluated to date and are the focus of this chapter. Preliminary observations by our collaborators suggest that **P3** has poor aqueous solubility. While **P3** was found to have greatest reduction in oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in an *in vitro* isogenic model of OAC radioresistance, its hydrochloride salt, **P4**, demonstrated superior reduction in OCR and ECAR versus **P3** *in vivo*, in zebrafish models, indicating that poor water solubility could be a limiting factor for translation of *in vitro* towards *in vivo* biochemical activity.⁴⁵¹ Modification of **P3**'s structure is necessary to achieve the required aqueous solubility for pre-clinical *in vivo* models.

8.2 Solubility issues with **P3** during *in vivo* pre-clinical animal models

Poor solubility of **P3** in a variety of vehicles trialed during animal xenograft models carried out by Dr. Susan Kennedy at Xenopat, Barcelona (January 2019) has been noted. (Figure 8.1) **P3** dissolved in 99% corn oil: 1% DMSO. While corn based vehicles are widely used for delivery of lipophilic agents during drug development, use of an aqueous based vehicle is more desirable for progression toward clinical trials.^{757, 758}

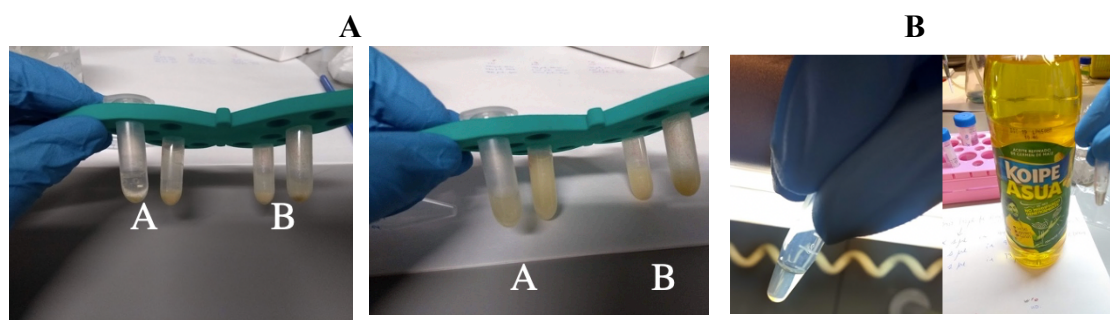
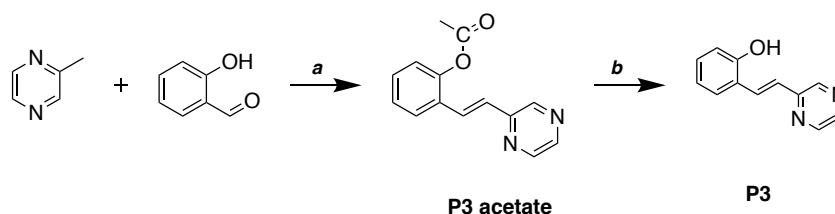


Figure 8.1: **A: (Left)** **P3** suspended in vehicle A (10% DMSO, 30% EtOH, 60% PBS) and vehicle B (10% DMSO, 30% EtOH, 60% H₂O) at 1 mg/mL. **(Right)** Post vortex **B: P3** dissolved in 99:1 non pharmaceutical grade corn oil: DMSO

8.3 Synthesis of **P3** using conventional reflux

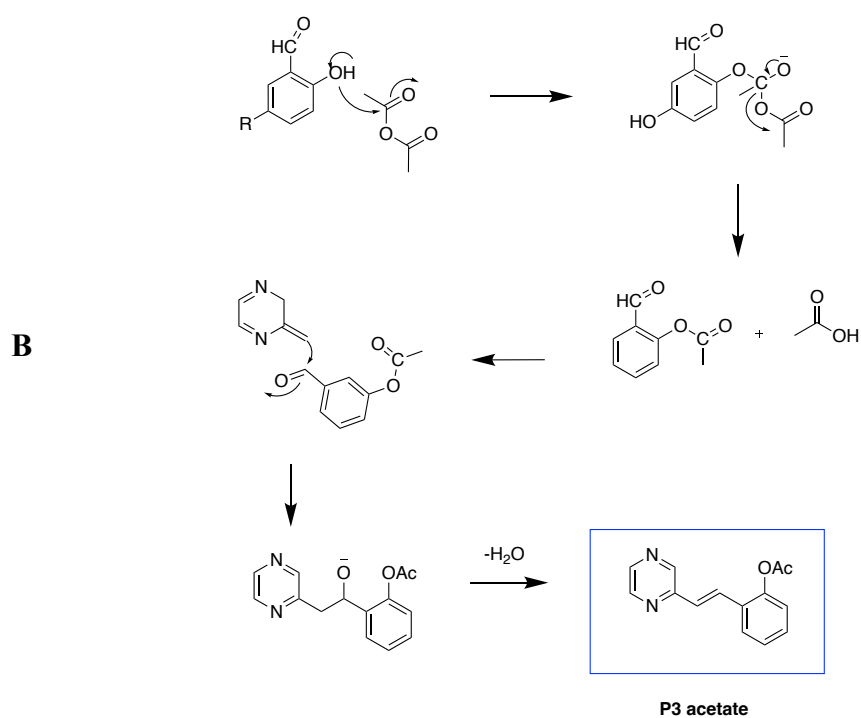
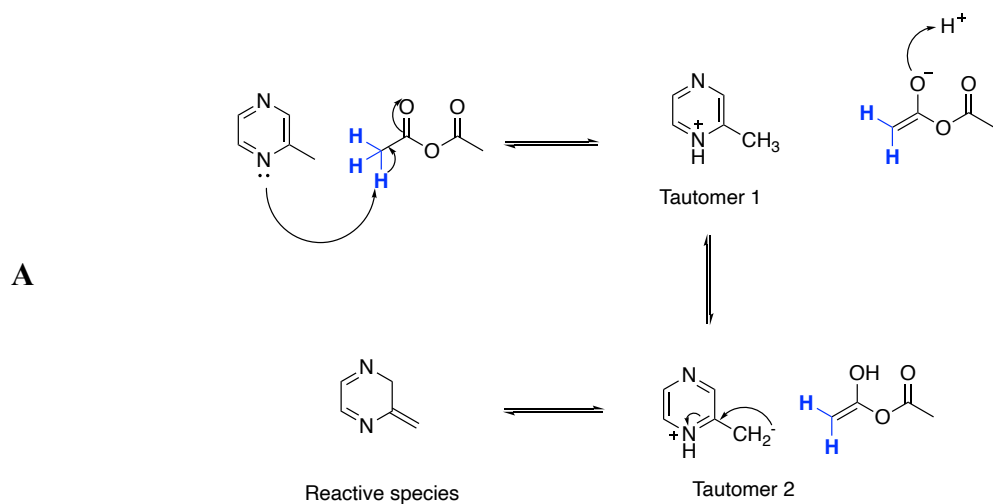
P3 was synthesised using the Perkin condensation reaction with 2-methylpyrazine and salicaldehyde in acetic anhydride. Scheme 8.1 details the synthesis of **P3** using the Perkin

condensation reaction while Scheme 8.2 illustrates the proposed Perkin condensation reaction mechanism *via* a 2-methylene reactive species. **P3** was isolated in poor yield of < 25% which is explained by limited tautomerisation potential for 2-methylpyrazine (**A**, Scheme 8.2). The pK_a of pyrazine is 0.7. Tautomerisation illustrated in **A**, Scheme 8.2 is therefore not favoured.



Scheme 8.1: Perkin condensation for synthesis of **P3**. **a**: Acetic anhydride, 180 °C, 4 days **or** microwave on standard power, 160 °C, 5 h. **b**: 1:1 EtOAc/ EtOH with 1M NaOH

The ^1H NMR spectra of **P3** confirms the *trans* configuration with presence of the *E* alkene doublets resonating at δ 8.03 and 7.37 ppm with ^3J values of 16 Hz which are characteristic of *trans* alkenes, corresponding to H_1 and H_2 respectively. The presence of a broad singlet at δ 10.03 ppm confirmed successful hydrolysis of the acetate group to afford the free phenolic moiety of **P3** (Figure 8.2).



Scheme 8.2: Proposed mechanism for the Perkin condensation reaction *via* **A**: tautomerisation of 2-methylpyrazine to form a methylene reactive species and **B**: reaction of 2-methylene reactive species with salicylaldehyde to afford P3 acetate (blue box).

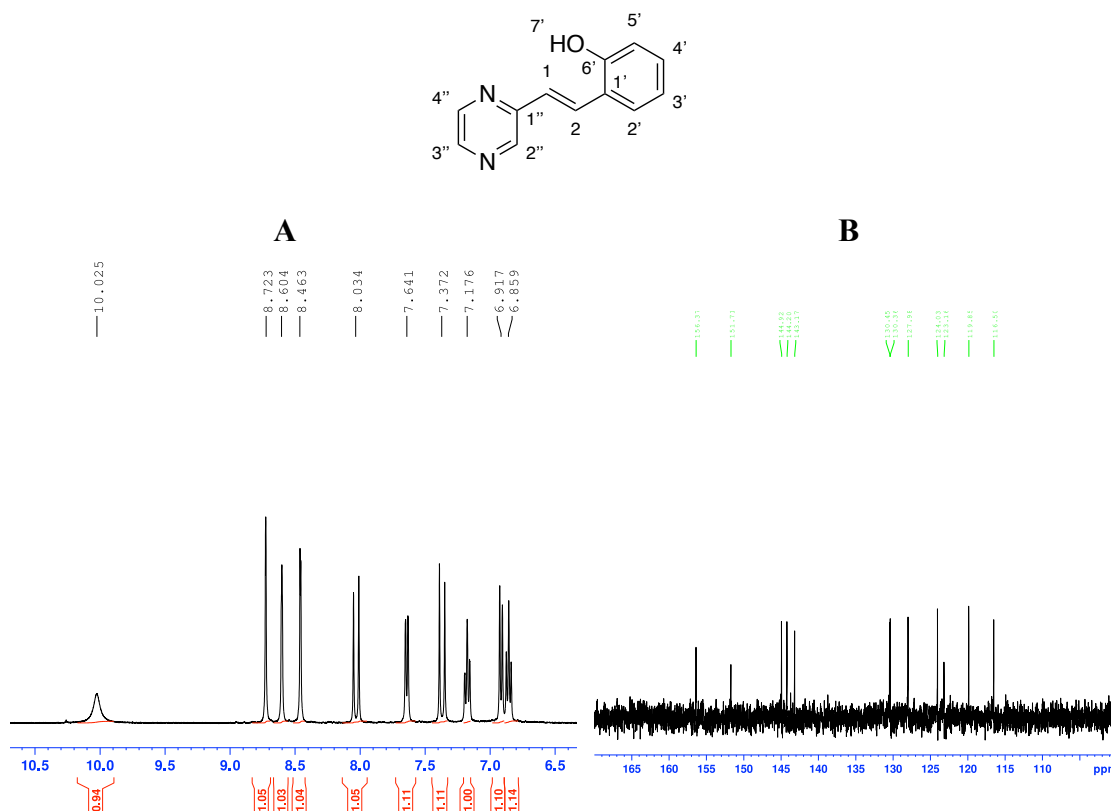


Figure 8.2: **A:** ¹H NMR of **P3** in DMSO-*d*₆ at 400 MHz. **B:** ¹³C NMR of **P3** in DMSO-*d*₆ at 100 MHz.

8.4 Synthesis of **P3** via MAOS

Conventional reflux for the Perkin condensation route to **P3** required long reaction times of 5-7 days and in poor yield (20-25 %). MAOS was therefore employed with aim of decreasing the reaction time and increasing crude % yield. MAOS was carried out on a 10 mmol scale for 5 hours at 160 °C with a reduced volume of acetic anhydride (5 mL). **P3 acetate** (Scheme 8.2) was isolated in 15% yield with minimal evidence of the hydrolysed phenolic **P3** on crude NMR spectra. This compared to observations using conventional reflux which yielded a mixture of both **P3 acetate** and **P3**. The shorter reaction time employed using MAOS, despite the lower yield, enabled rapid reaction repetition for scale up of **P3** synthesis when compared to conventional reflux

8.5 XRD analysis of **P3**

P3 was recrystallised as an orthorhombic system from a saturated solution of 1:1 *n*-hexane/ethyl acetate over 24 hours. XRD analysis confirmed the *trans* alkene bond of 1.33 Å (Figure 8.3). The XRD structure suggests a potential reason for the poor observed aqueous solubility. Intermolecular hydrogen bonding is observed between **P3**'s phenolic hydroxyl and the pyrazine N14 nitrogen (Figure 8.3). This may prohibit hydrogen bonding with solvent and therefore limit solvation (**B**, Figure 8.3).

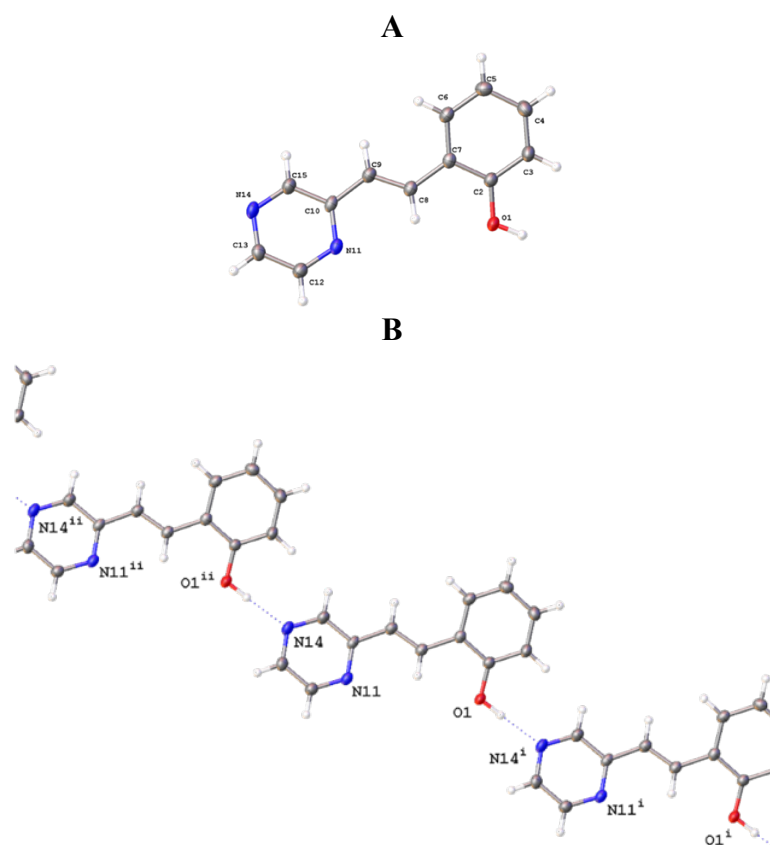


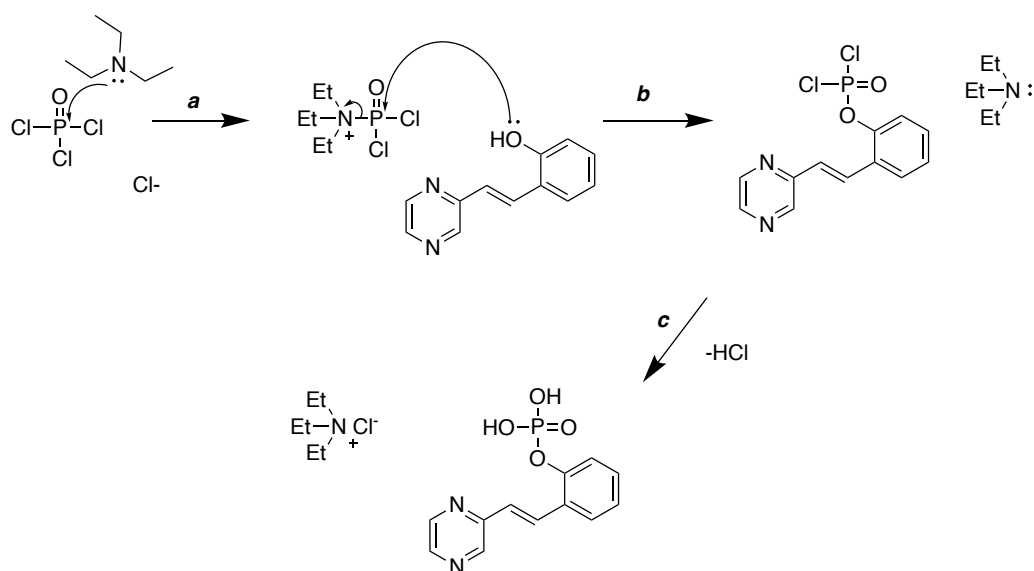
Figure 8.3: **A**: Molecular structure of **P3** with atomic displacement shown at 50% probability. **B**: Detail of the intermolecular hydrogen bonding between molecules of **P3** (dotted lines) with atomic displacement shown at 50% probability.

8.5.1 Phosphate prodrug synthesis for **P3**

The phosphate prodrug of **P3** was selected as a strategy to overcome poor dissolution and underperformance *in vivo* when compared to **P3**'s *in vitro* activity.⁴⁵¹ Two alternative synthetic strategies were explored for synthesis of **P3** phosphate (**P3P**) (Scheme 8.3 and 8.4).

8.5.2 Synthesis of **P3P** using phosphorous oxychloride

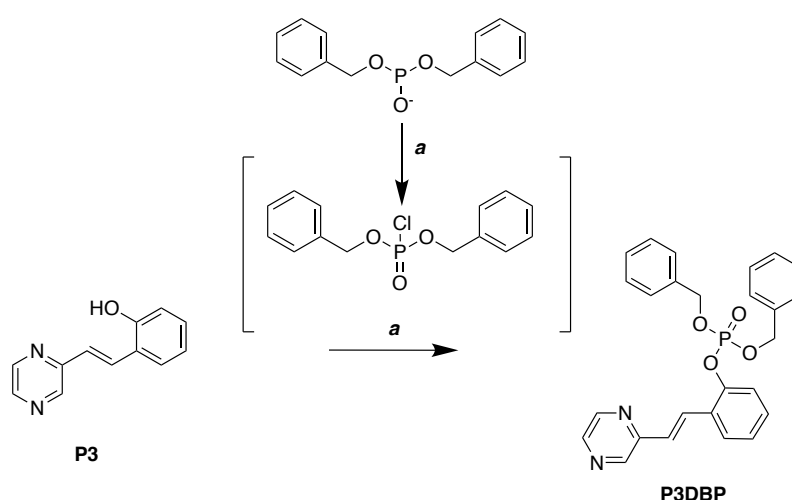
A one pot synthesis for the isolation of **P3P** involving the use of phosphorous oxychloride (POCl_3) was trialled (Scheme 8.3). The reaction of phenolic **P3** proceeded with POCl_3 through nucleophilic attack of the phenolic **P3**'s oxygen with the phosphorous atom of POCl_3 to yield a phosphorochloridate intermediate *via* a bi-molecular SN_2P mechanism. Typically a base is typically employed for activation of POCl_3 . TEA has demonstrated success for activation of POCl_3 .⁷⁵⁹ The positively charged intermediate is a more reactive phosphorylating reagent than native POCl_3 . Additionally, TEA fixes liberated HCl in the form of a corresponding chlorhydrate ($\text{TEA} \cdot \text{HCl}$). An excess of TEA is typically employed to scavenge HCl to prevent the formation of byproduct chloroalkanes. The precipitation of this chlorohydrate in appropriate solvent promotes formation of phosphoric acid. Water is employed to hydrolyse the phosphorochloridate intermediate to the free phosphoric acid as the last step⁷⁵⁹ (Scheme 8.3). Successful formation of the **P3P** was determined by HRMS, ^1H NMR and ^{31}P NMR, however as a TEA complex in ratio 98:2 TEA: **P3P**, determined using qNMR. Due to complexities associated with de-salting of small yields, the use of dibenzyl phosphite as a phosphorylating reagent was explored.



Scheme 8.3: Reaction of **P3** with POCl_3 . **a:** TEA activates POCl_3 to form the reactive TEA- POCl_3 complex in anhydrous THF **b:** **P3** reacts with TEA- POCl_3 complex yielding the phosphoro chloridate intermediate and TEA. **c:** Water hydrolyses the phosphorochloridate to the free phosphoric acid.

8.5.2.1 Synthesis of **P3P** using dibenzylphosphite

Reaction of **P3** with dibenzylphosphite *via* the Atherton-Todd reaction afforded a dibenzyl ether phosphate of **P3** as **P3DBP** in good yield (60%) as a yellow oil (Scheme 8.4). Confirmation of dibenzyl phosphite phosphorylation was observed on ^1H NMR with benzyl CH_2 protons integrating as 4H with two singlets at δ 4.65 and 4.63 ppm. 10 additional aromatic protons were observed as a complex multiplet at δ 6.83 ppm of **P3** dibenzyl phosphate's benzyl rings (**A**, Figure 8.4). The ^{31}P NMR spectrum demonstrate the presence of a single phosphate only at δ -6.6 ppm, (**B**, Figure 8.4) confirming the purity of desired target.



Scheme 8.4: Synthesis of **P3** dibenzyl phosphate (**P3DBP**). CCl_4 , 0°C , N_2 , MeCN, 15 minutes with **P3**. Then DMAP, DIPEA, dibenzyl phosphite at 0°C . Then RT for 24 hours.

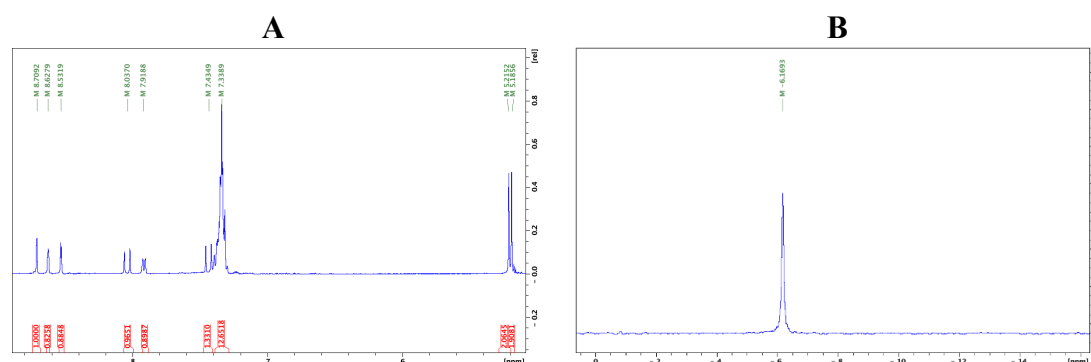


Figure 8.4: **A:** ^1H NMR spectrum for **P3DBP** in $\text{DMSO}-d_6$ at 400 MHz. **B:** ^{31}P for **P3DBP** in $\text{DMSO}-d_6$ at 162 MHz

8.5.2.2 Synthesis of P3 dimethyl phosphate (**P3DMP**) using dimethyl phosphite

Dimethyl phosphite was also explored as a phosphorylating reagent also *via* the Atherton-Todd reaction as illustrated in Scheme 8.4. The **P3** dimethyl phosphate product (**P3DMP**) was isolated in 41-50% yield as a brown oil. The dimethyl protons of the phosphate moiety were observed as singlets at δ 3.84 and 3.80 ppm (**A**, Figure 8.5). The presence of one major phosphorylated product is confirmed by the ^{31}P NMR spectrum with one resonance signal at δ -2.44 ppm (**B**, Figure 8.5). However after storage at 2-8 °C for ~6 months, **P3DMP** (brown oil) had transformed into a powder. NMR analysis demonstrated transformation of the original NMR spectra. A new and major ^{31}P resonance was observed at δ -3.94 ppm accompanied by minor presence of the original δ -2.94 ppm resonance (**B**, Figure 8.5). LC-MS demonstrated presence of two compounds with molecular weight of the major degradation product identified as 293.00 ($[\text{M}+\text{H}^+]$). Spontaneous atmospheric hydrolysis of a single methyl group occurred during storage to yield the monomethyl phosphoric acid of **P3** (**C**, Figure 8.5). **P3DMP** was therefore not carried forward as a suitable phosphate prodrug of **P3** due to these stability issues. Instead focus was placed on debenzylation of **P3DBP** (Scheme 8.4 and Scheme 8.5) to yield the free phosphoric acid of **P3** (**P3P**). In the future optimal storage should be investigated followed by optimisation of dimethyl group demethylation.

8.5.2.3 Removal of dibenzyl groups from **P3DBP** using the McKenna reaction

A simple catalytic hydrogenation in the presence of palladium rapidly typically removes dibenzyl groups to afford free phosphoric acids. This has been achieved for the β -lactam derivatives of CA-4.³⁸⁰ However, the presence of **P3**'s *trans* alkene bond prohibits hydrogenation as a method for removal of dibenzyl groups which would otherwise hydrogenate the alkene to an alkane bond. In the synthesis of CA-4 phosphate, which also contains a *cis* double bond, the McKenna reaction was successfully employed to produce the phosphoric acid in excellent yields.³²⁰ The McKenna reaction is a widely known tool for formation of organophosphorus acids from esters.⁷⁶⁰ However it is water sensitive and accompanied by a plethora of side reactions and decomposition products. **P3P** was not isolated using the McKenna reaction (**b**, Scheme 8.5).

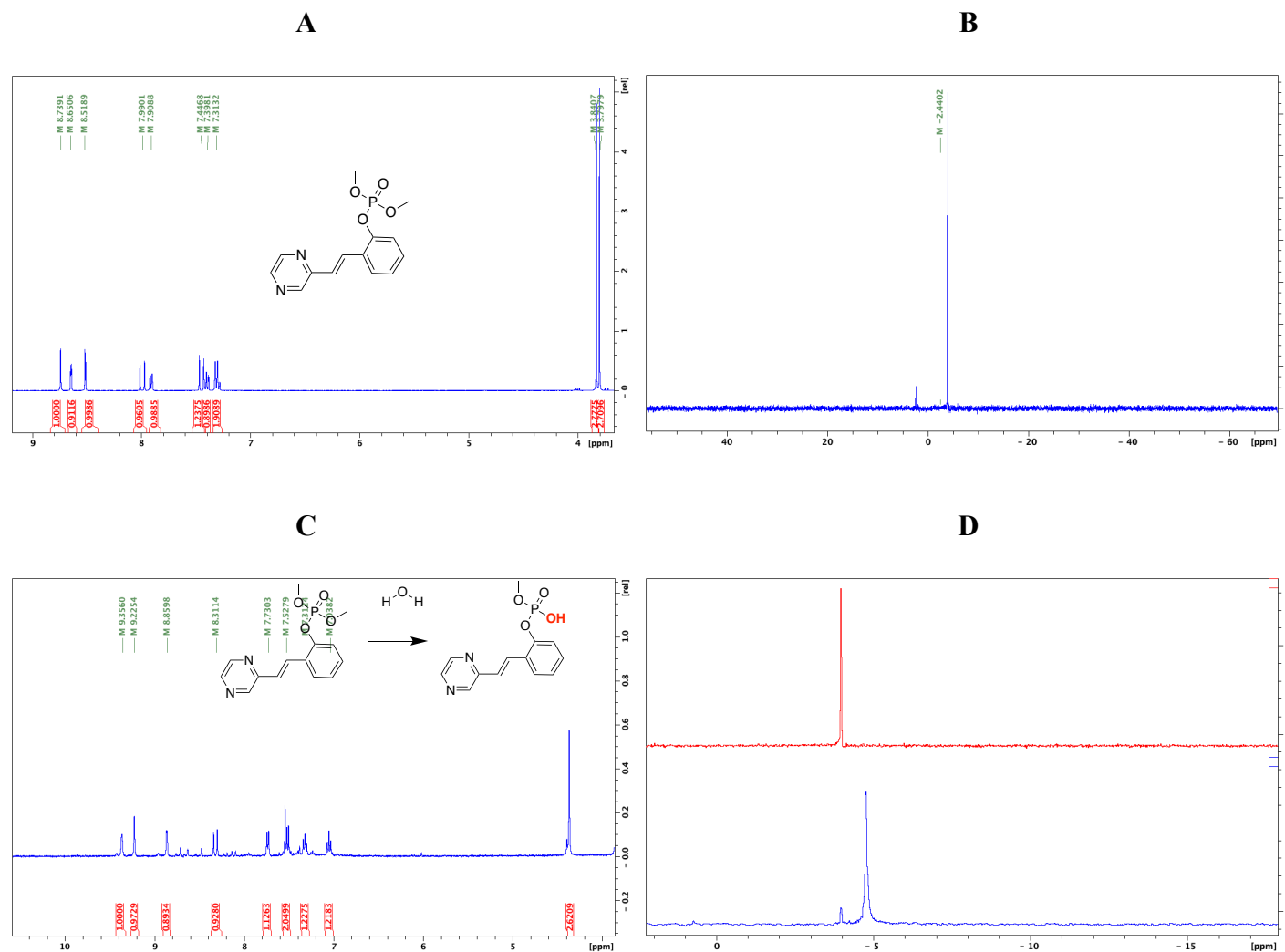
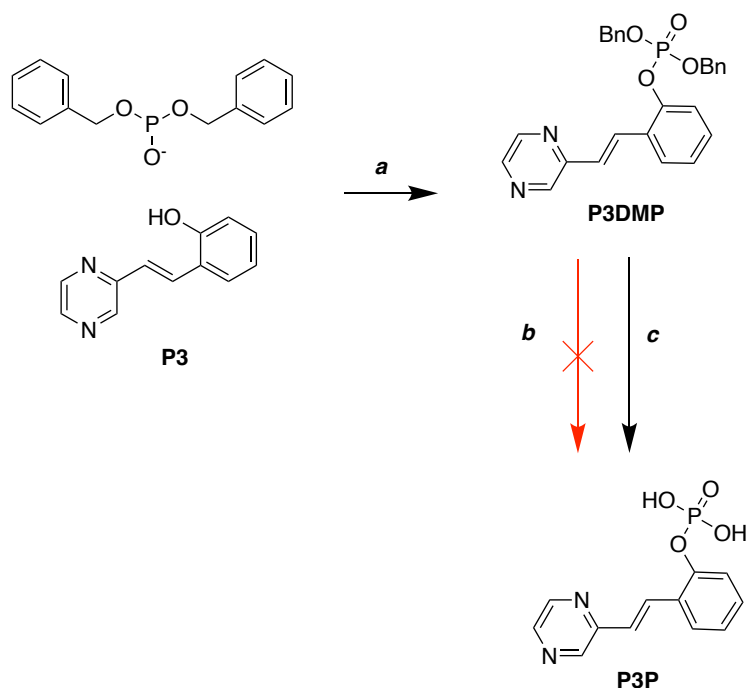


Figure 8.5: **A:** ^1H NMR spectrum of **P3MP** in $\text{DMSO}-d_6$ at 400 MHz. **B:** ^{31}P NMR spectrum of **P3DMP** at 162 MHz. **C:** Spontaneous atmosphere hydrolysis of **P3DMP** to **P3** monomethyl phosphate and ^1H NMR in $\text{DMSO}-d_6$ at 400 MHz after 6 months of storage. **D:** ^{31}P NMR spectra of degraded **P3** monomethyl phosphate (blue) versus **P3DMP** in >99% purity (red)

8.5.2.4 Removal of dibenzyl groups using boron tribromide

Boron tribromide (BBr_3) is an inexpensive and effective alternative to trimethylsilane derivatives for dealkylation of dialkyl phosphonates that contain functional groups.

A modified version of a reported reaction was explored for debenzylation of **P3DBP** (**c**, Scheme 8.5)⁷⁶¹ and purified using size exclusion chromatography with Sephadex® G-10 medium for isolation of **P3P** in 40% yield.



Scheme 8.5: **a**: Synthesis of **P3DBP** as per Scheme 8.4 **b**: McKenna reaction for debenzylation. NaI , Me_3SiCl , 0°C , N_2 **c**: Debenzylation to **P3P** using BBr_3 (1M in hexane), anhydrous toluene, -10°C , then 80°C for 2h, N_2 . Then MeOH , 1 h. (Yield 40%)

8.6 Aqueous solubility of **P3** and **P3P**

The aqueous solubility of **P3** and **P3P** was measured using the shake-flask method, a procedure comprising of sample preparation, equilibration in aqueous buffer, separation of solid precipitates from solution containing the dissolved drug and analysis of the dissolved content in the filtered solution using HPLC.⁷⁶² Shake flask experiments were carried out in line with WHO guidance.⁷⁶³ All measurements are made in triplicate and reported at mg/mL . All shake flask experiments were placed on a mechanical (orbital) shaker (Mini gyro-rocker, SSM3, Stuart) with a stirring speed of 70 rpm for 24 hours.

After 24 hours the suspensions were filtered through a 0.22 micron filter to remove undissolved drug and the filtrate was subsequently analysed by HPLC. HPLC studies were carried out at the λ of max absorption of 348 nm and 334 nm for **P3** and **P3P** respectively. A series of standard serial solutions for **P3** and **P3P** were prepared from a stock solution (0.5 mg/ mL) in 70:30 MeCN: H₂O. Calibration curves for **P3** and **P3P** were constructed within linear range of absorbance (**A** and **B**, Figure 8.6). The solubility of **P3P** was measured as 0.31 mg/ mL compared to 0.00005 mg/ mL for **P3**. Derivatisation of **P3** to its phosphate prodrug **P3P** resulted in a 6200-fold increase in aqueous solubility.

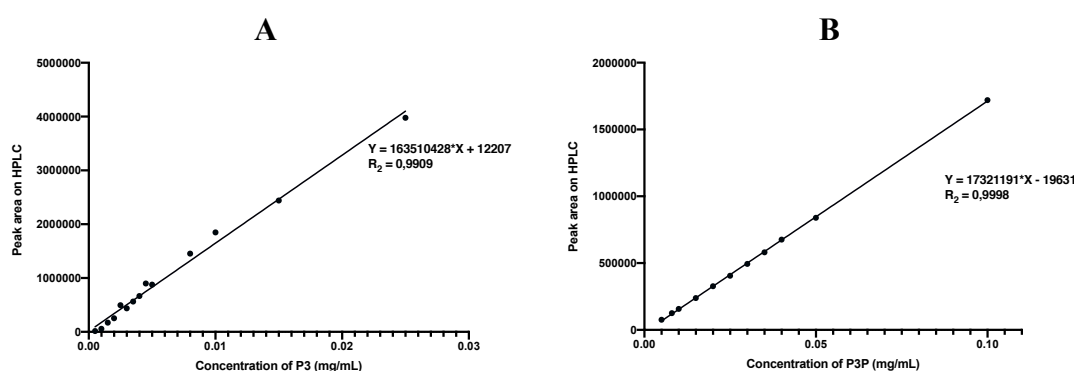


Figure 8.6: Calibration curves used for solubility measurements for **A: P3** **B: P3P**

8.7 Measurement of logP for **P3** and **P3P**

LogP measurements were carried out comparing **P3** to **P3P** using the procedure described in Chapter 4. These values were compared to values obtained using the Swiss ADME webtool and the estimated cLogP values obtained from ChemDraw. Both methods for estimating logP values were comparable to experimentally derived values of 1.55 and -2.35 for **P3** and **P3P** respectively (Table 8.1). The increase in hydrophilicity and solubility imparted by addition of the phosphate for **P3P** is reflected by the decrease in logP by > 3 units from 1.55 for **P3** to -2.35 for **P3P** (Table 8.1).

Table 8.1: Experimentally measured logP values for **P3** and **P3P** compared to values obtained using Swiss ADME webtool and clogP.

Code	cLogP (ChemDraw)	Swiss ADME derived logP	Experimentally determined logP	Measured solubility (mg/ mL)
P3	1.17	1.80	1.55	0.000051
P3P	-0.17	0.81	-2.35	0.31

8.8 Chemical stability of *P3* versus *P3P*

The stability of **P3** and **P3P** was analysed in phosphate buffers at room temperature at three pH values (acidic, neutral and basic pH with buffer compositions described in Chapter 9) over a period of 1-7 days. Both compounds were extremely stable at all three pH values. >90% of the compound remains for all time points and all pH ranges at 7 days (Figure 8.7).

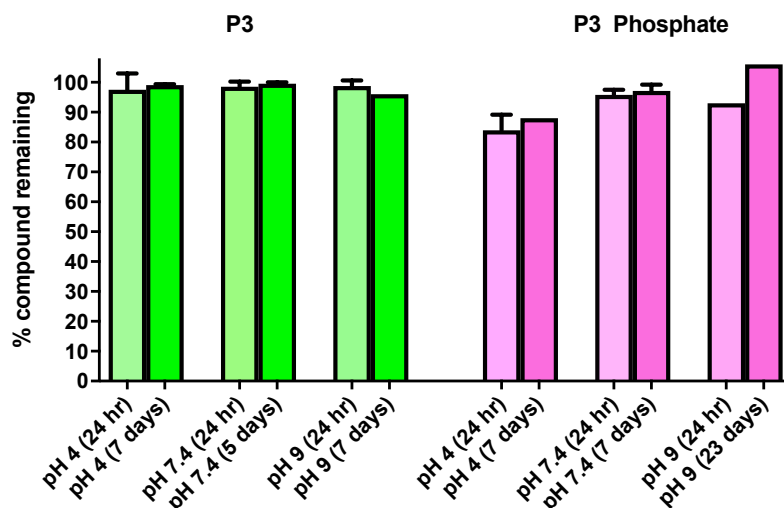


Figure 8.7: Stability of **P3** and **P3P** at 24 hr and 7 days in phosphate buffers BP at pH values of 4, 7.4 and 9. Two exceptions noted (**P3** pH 7.4 measured at 5 days and **P3P** at pH 9 measured at 23 days due to Covid-restricted lab access).

8.9 Alkaline phosphatase mediated hydrolysis of *P3P*

Alkaline phosphatases (AP) are ubiquitous enzymes and are typically anchored to the outside surface of cellular plasma membranes and catalyse the hydrolysis of phosphate groups from a variety of substrates when in alkaline environments.^{764,765} This experiment aimed to demonstrate the rapid dephosphorylation of **P3P** to **P3** in the presence of AP.

A stock solution of **P3P** (2.78 mg/1 mL in borate buffer adjusted to pH 7.4) was prepared and diluted with borate buffer to make a 0.3 mM stock solution. 10 mL of AP solution (Merck P7640-500MG) was prepared as 0.5 mg/mL in distilled water. AP stop solution (Merck A5852-100ML) employed to stop AP enzyme activity, was prepared by reconstitution of the dry powder in 100 mL of distilled water. The solutions were pre-heated separately for 15 min at 37 °C to facilitate optimal AP activity. HPLC vials were prepared containing reconstituted AP stop solution (50 µL). The experiment was performed in triplicate. Replicate 1 involved addition of 5 mL of 0.5 mg /mL AP solution to 10 mL of **P3P** (1:2 AP: **P3P** stock solution) while replicates 2 and 3 added 5 mL of 0.5 mg /mL AP solution to 5 mL of **P3P** stock solution (1:1 AP: **P3P** stock solution). The reaction solution (200 µL) was removed at indicated time points and added to each of the prepared HPLC vials containing the stop solution. Relative peak areas from HPLC analysis for **P3** and **P3P** were used to determine % conversion of **P3P** by AP to parent **P3**.

At higher enzyme concentrations (1:1 volume of AP: **P3P**), 100% dephosphorylation to **P3** was observed by 5 seconds (**A**, Figure 8.8). At lower enzyme concentration (1:2 volume AP: **P3P**) conversion was marginally slower. 100% conversion was observed between 30 seconds to 1 minute (**B** and **C**, Figure 8.8). The phosphate prodrug approach may serve as an effective strategy of ensuring aqueous dissolution of **P3** while maintaining **P3**'s properties due to its rapid conversion to parent in by enzymes anticipated to be present *in vivo*.

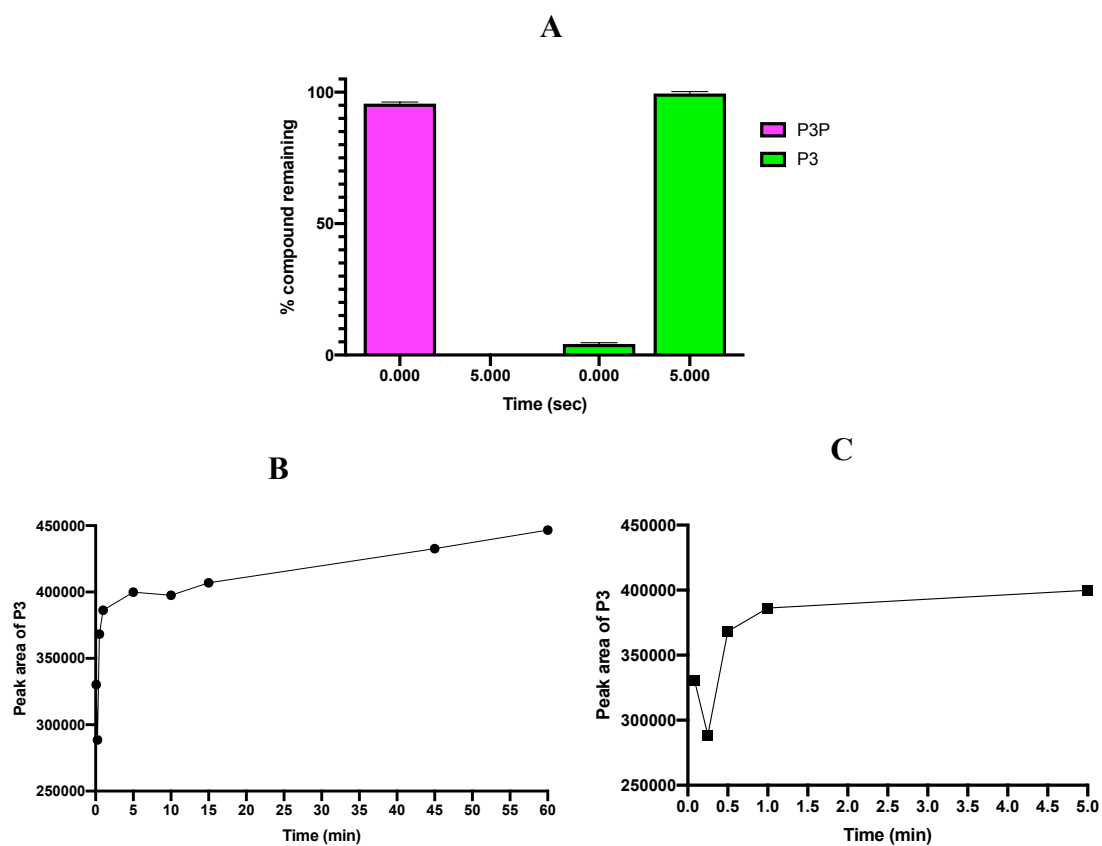


Figure 8.8: Conversion of **P3P** to **P3** by alkaline phosphatase at 37 °C **A**: 5 mL of AP solution (25 DEA units) was added to 5 mL of **P3P** of 0.3 mM stock solution **B**: 5 mL of AP solution (25 DEA units) was added to 10 mL of **P3P** of 0.3 mM stock solution **C**: Enlarged area of **B** from 0-5 minutes

8.10 Comparison of antiproliferative activity for **P3** versus **P3P** in breast cancer cells

The antiproliferative activity of **P3** and **P3P** were compared using in-house cell viability assays (AlamarBlue™) at three concentrations in two breast cancer cell lines; MCF-7 (**A**, Figure 8.9) and MDA-MB-231 cells (**B**, Figure 8.9). In MCF-7 cells, **P3** demonstrated minimal antiproliferative activity at 1 and 10 μM . Significant antiproliferative activity was however demonstrated at 100 μM for **P3** with a p value of <0.001 (**A**, Figure 8.9). Significant antiproliferative activity was not observed for **P3** at any concentration in MDA-MB-231 cells (**B**, Figure 8.9). A significant difference is observed between % of viable cells treated with **P3** (10 μM) versus **P3P** (10 μM) in both MCF-7 cells ($p < 0.001$, **A**, Figure 8.9) and MDA-MB-231 cells ($p < 0.05$, **B**, Figure 8.9). **P3P** demonstrated superior antiproliferative activity compared to **P3**. *In vitro* IC_{50} values were measured as 2.56 μM in MCF-7 cells and 24.7 μM in MDA-MB-231 cells for **P3P**. **P3P** is therefore approximately 10-fold more potent in MCF-7 cells compared to MDA-MB-231 cells based on its aforementioned IC_{50} values. Future work must determine whether **P3P** exerts its biological activity through the ER, present in MCF-7 but not MDA-MB-231 cells, which may cause significant variation in antiproliferative activity between the two breast cancer cell lines.

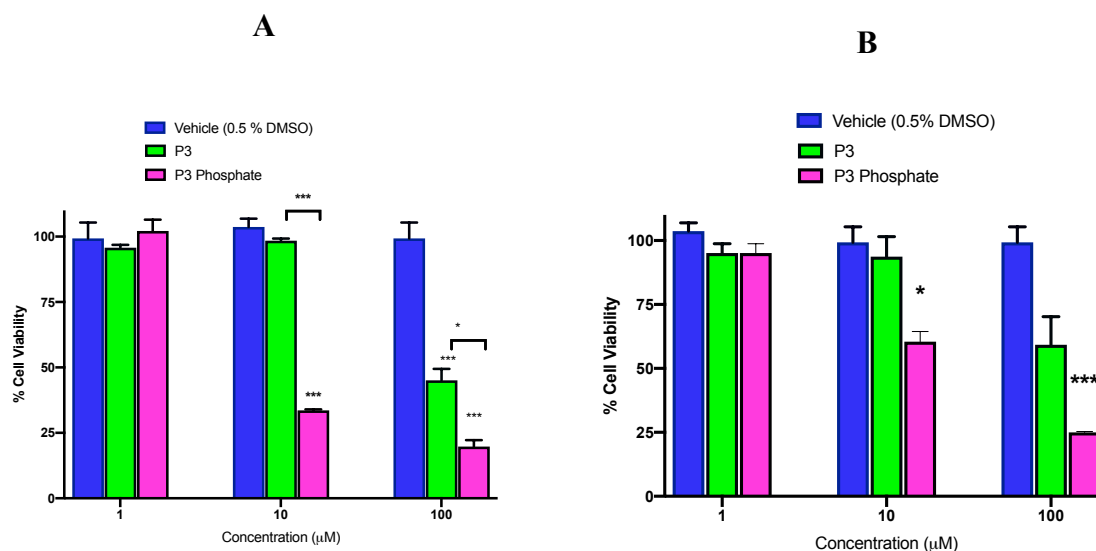


Figure 8.9: Antiproliferative activity of prodrug **P3P** (pink) versus **P3** (green) in **A**: MCF-7 cells. **B**: MDA-MB-231 cells. Values represent average of three independent replicates performed in triplicate on three independent occasions. Statistical analysis involved using multiple t-test comparisons corrected for multiple comparisons using the Holm-Sidak method to establish statistical significance with respect to control. * p-value <0.05 . *** p-value <0.001 .

8.11 Cell cycle studies for **P3** and **P3P** in MDA-MB-231 cells

To rationalise the increase in antiproliferative activity for **P3P** versus **P3**, cell cycle studies were carried out in MDA-MB-231 cells. Due to limited availability of **P3P** the data presented in Figure 8.10, Table 8.2 and Table 8.3 represent preliminary data from one replicate (n=1). **P3P** appeared to induce G₂/M phase arrest in MDA-MB-231 cells (Table 8.3) in contrast to **P3** which has no effect at any concentration compared to vehicle control and does not cause G₂/M phase arrest (Table 8.2). At 8 hours following treatment with **P3P** (20 µM) 47% of MDA-MB-231 cells had arrested in G₂/M phase which increased to 69% at 24 hours. (Table 8.3) Additionally, at 48 hours a rise in the % of sub G₁/G₀ cells was observed compared to vehicle control. For example 12% of cells treated with **P3P** (25 and 100 µM) had become apoptotic by 48 hours (Table 8.3). Future work will involve re-synthesis of **P3P** and confirmation of cell cycle findings *i.e* the effect of **P3P** on G₂/M cell population in a time and dose dependent manner.

Table 8.2: % of MDA-MB-231 cells in each stage of cell cycle when treated with **P3** (25, 32.5, 50 or 100 μM) for 8, 24 or 48 h. Vehicle = DMSO 0.5% v/v.

G₁/G₀	Vehicle	25 μM	32.5 μM	50 μM	100 μM
8h	52.52	52.63	51.50	50.95	53.76
24h	49.43	52.32	47.41	55.85	54.46
48h	53.37	63.91	64.01	57.20	53.00
S	Vehicle	25 μM	32.5 μM	50 μM	100 μM
8h	10.08	9.98	11.91	14.28	13.49
24h	10.92	9.85	12.54	10.24	8.59
48h	12.12	8.73	8.23	11.01	10.91
G₂/M	Vehicle	25 μM	32.5 μM	50 μM	100 μM
8h	36.02	36.32	34.93	32.90	30.18
24h	37.21	36.24	37.64	32.58	29.73
48h	32.04	25.99	26.45	29.23	31.72
Sub G₁/G₀	Vehicle	25 μM	32.5 μM	50 μM	100 μM
8h	0.93	0.83	1.11	1.09	1.60
24h	1.12	0.78	0.69	0.63	1.12
48h	0.80	0.81	0.76	2.26	4.74

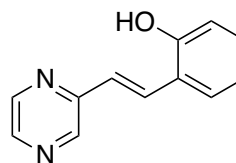
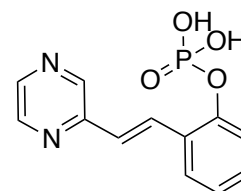


Table 8.3: % of MDA-MB-231 cells in each stage of cell cycle when treated with **P3P** (10, 20, 25, 32.5, 50 or 100 μM) for 8, 24 or 48 h. Vehicle [0.6% v/v ethanol/dH₂O].

G₁/G₀	Vehicle	10 μM	20 μM	25 μM	32.5 μM	50 μM	100 μM
8h	52.52	49.87	47.18	36.59	33.37	31.73	35.78
24h	49.43	60.97	36.50	20.22	15.07	24.26	34.00
48h	53.37	56.57	28.28	20.78	24.78	24.11	23.04
S	Vehicle	10 μM	20 μM	25 μM	32.5 μM	50 μM	100 μM
8h	10.08	11.10	12.63	14.64	12.91	11.97	12.65
24h	10.92	6.72	8.80	7.64	6.86	8.25	11.97
48h	12.12	8.55	11.89	7.45	10.45	8.99	8.69
G₂/M	Vehicle	10 μM	20 μM	25 μM	32.5 μM	50 μM	100 μM
8h	36.02	36.61	37.35	47.41	52.07	54.63	49.80
24h	37.21	29.39	45.68	68.57	75.44	63.54	47.30
48h	32.04	31.27	52.22	61.07	59.07	57.10	57.80
Sub G₁/G₀	Vehicle	10 μM	20 μM	25 μM	32.5 μM	50 μM	100 μM
8h	0.93	2.05	2.38	1.12	1.12	1.01	0.96
24h	1.12	2.72	9.77	3.42	2.93	3.75	7.01
48h	0.80	3.04	8.46	11.57	3.21	9.95	11.56



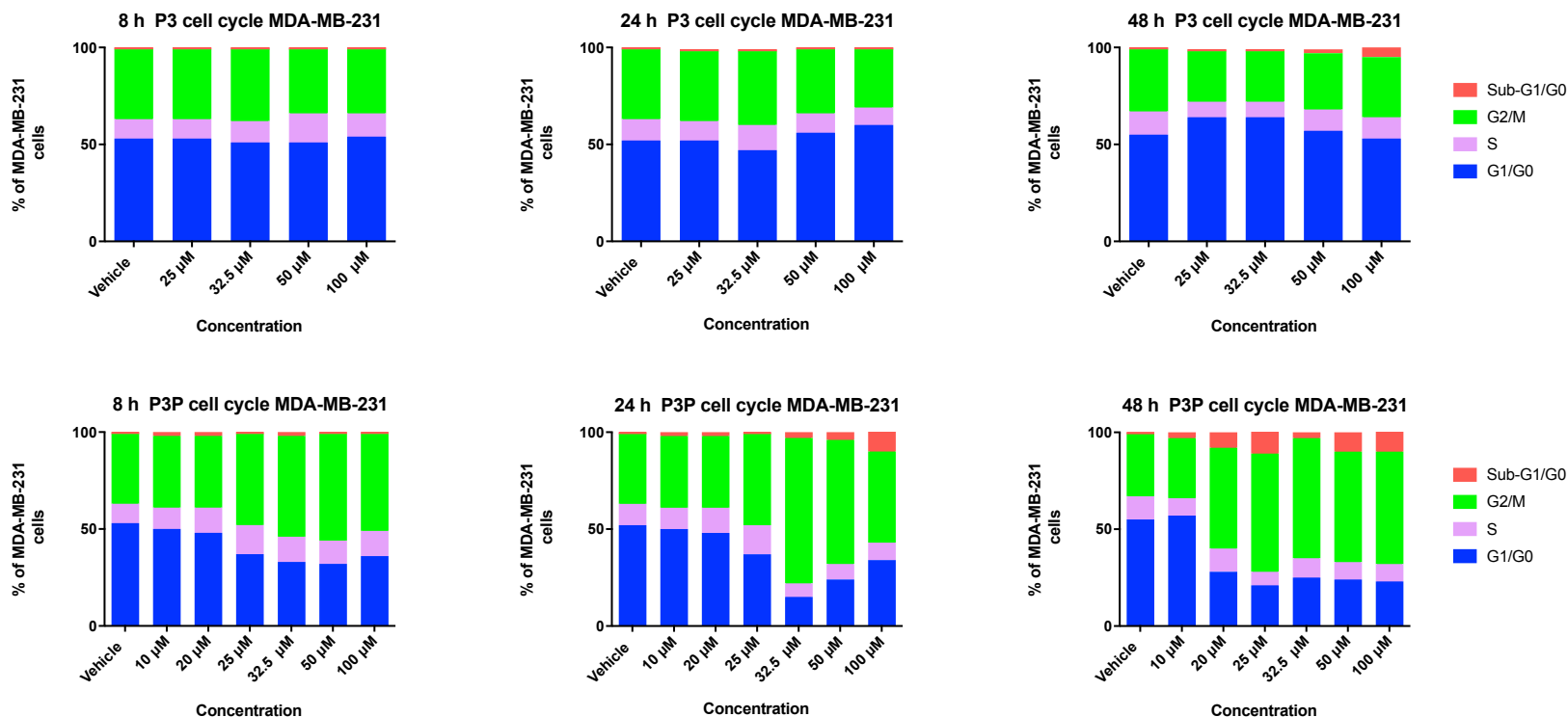


Figure 8.10: Effects of **P3** and **P3P** on the cell cycle in MDA-MB-231 cells. For **P3** cell cycle experiments, cells were treated with vehicle [DMSO 0.5% (v/v)] or **P3** at concentrations of 25, 32.5, 50 or 100 μ M. For **P3P** cell cycle experiments, cells were either treated with vehicle (0.6% v/v ethanol) or **P3P** at 10, 20, 25, 32.5, 50 or 100 μ M. Cells were treated for 8, 24 or 48 h. Cells were then fixed, stained with PI and analysed by flow cytometry. Cell cycle analysis was performed on histograms of gated counts per DNA area (FL2-A). The number of cells with <2N (sub-G₁), 2N (G₀/G₁), and 4N (G₂/M). DNA content was determined using the BD accuri C6 flow cytometer. Values represent the mean for one experiment. (n=1)

8.12 Molecular modelling for *cis* and *trans* **P3**

P3 has been synthesised in this thesis as the *trans* form. The pyrazine A and phenolic B rings of *trans* **P3** have similar *trans* orientation to the A and B rings of CA-4's inactive *trans* configuration (Figure 8.11).⁴⁹³ *Cis* CA-4 is active as a CBSI while *trans* CA-4 is not. A computational study was therefore carried out, modelling the *cis* isomer of **P3** in order to ascertain potential microtubule depolymerising potential with CBS affinity of *cis* **P3** comparable to *cis* CA-4 (Figure 8.12). This computational study was carried out in *MOE* using the docking protocol optimised for the docking of β -lactam enantiomers described in Chapter 5 (Figure 5.7).

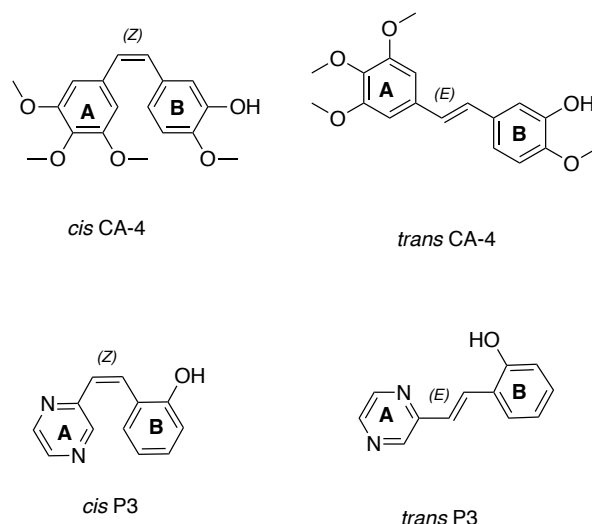


Figure 8.11: Structures of *cis* and *trans* CA-4 and **P3**

Cis and *trans* **P3** were modelled at the CBS of 1SAO. Computational studies carried out for *cis* and *trans* **P3** indicate that *trans* **P3** docks unfavourably at the CBS and is therefore unlikely to have *in vitro* microtubule depolymerising activity (cyan, **A** and **B**, Figure 8.12), further explaining its minimal anti-proliferative activity observed in breast cancer cells (Figure 8.9). In comparison, *cis* **P3** (pink, **A** and **B**, Figure 8.12) closely aligns with DAMA-colchicine (green, **A** and **B**, Figure 8.12). *Cis* **P3** maintains many major interactions at CBS of 1SAO as described for CA-4 in Chapter 5.^{337, 338, 669} The pyrazine A ring of *cis* **P3** (Figure 8.11) hydrogen bonds with the SH of Cys β 241 within the β subunit of the CBS (blue dotted line, **A**, Figure 8.12). This interaction is known to be crucial for stabilising *cis*-CA-4 at the CBS and is imperative for microtubule destabilising activity as discussed previously in Chapter 5.³⁴² The pyrazine ring of *trans* **P3**

predominantly interacts with the α subunit of the tubulin heterodimer, in unfavourable orientation and this important interaction with Cys β 241 is not observed (**cyan**, **A** and **B**, Figure 8.12). The *cis* alkene configuration of **P3** is important for orienting **P3**'s pyrazine A ring at the β subunit of the CBS and in the same orientation as CA-4's trimethoxyphenyl A ring. It is plausible to suggest that the *cis* isomer of **P3** could have *in vitro* and *in vivo* microtubule depolymerisation activity due to this potential CBS affinity imparted by **P3**'s pyrazine A ring.

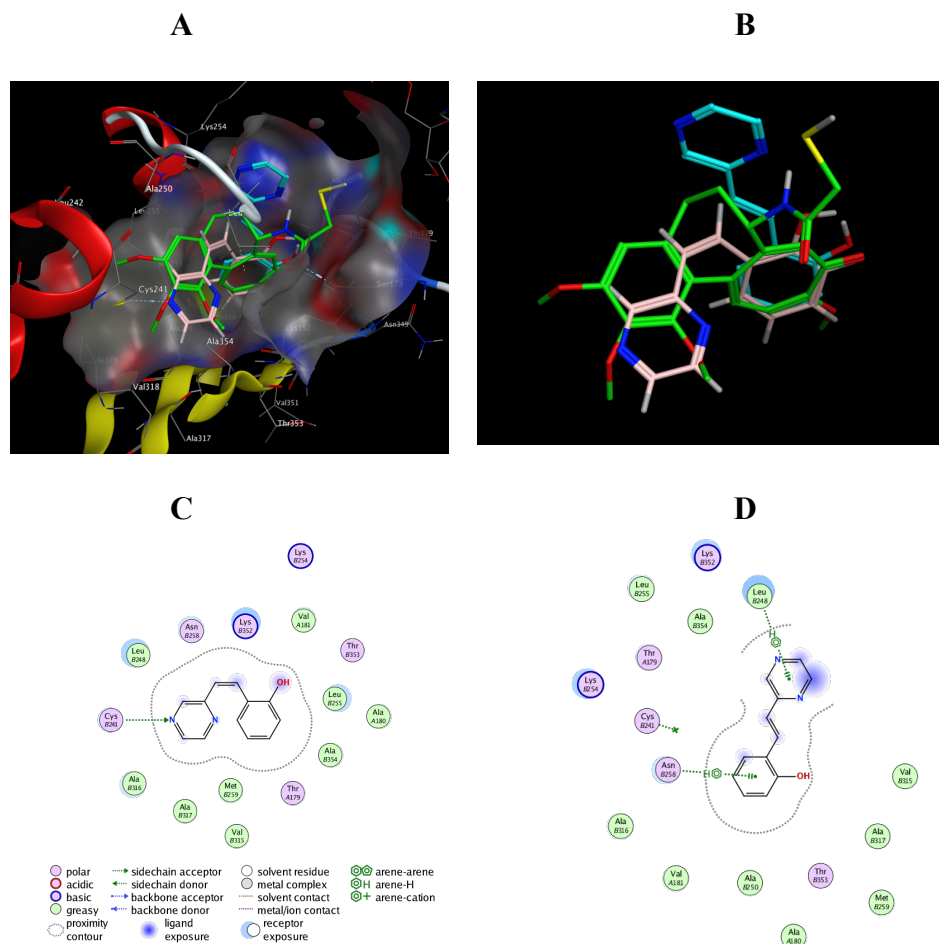


Figure 8.12: Molecular docking studies for *cis* and *trans* **P3** at the CBS of 1SAO **A: Cis P3** (pink) and *trans* **P3** (cyan). DAMA-Colchicine is shown in green. CBS binding pocket illustrated in grey. Hydrogen bonding shown as blue dotted lines. **B: Overlay of cis P3, trans P3** and DAMA-colchicine with receptor removed for clarity. **C: 2D Ligand-receptor interaction diagram for cis P3** and **D: trans P3** ligand interactions. Atom colours: Red: oxygen, Blue: Nitrogen, Yellow: Sulfur, Grey: Hydrogen.

Most recently it has been demonstrated that a series of CA-4 analogues modified in structure as anilines substituted at the 7-position of a 2-substituted-[1,2,4]triazolo[1,5-

a]pyrimidine nucleus had CBS activity as microtubule destabilisers. This demonstrated that the 3,4,5-trimethoxyaniline ring is not an essential requirement for CBS affinity.³⁷⁷ The importance of the 3,4,5-trimethoxyaniline ring is subject to debate in the literature.^{766, 767} This thesis aimed to further explore the anti-proliferative activity mediated by the pyrazine nucleus of **P3** in place of CA-4's 3,4,5-trimethoxyphenyl ring *via cis* restriction of **P3**'s A and B rings using the 2-azetidinone scaffold. This chemistry would yield a panel of **P3** β -lactams utilising the *cis* restriction approach applied successfully applied to β -lactams racemates of CA-4 to yield the combretazets panel, discussed in this thesis from Chapters 2-7.

8.13 Chemistry of **P3** β -lactam synthesis – ‘The Pyrazets’

‘The Pyrazets’ (Pyraz- and 2-azet-) were coined as a theoretical panel of *cis* restricted **P3** β -lactams, incorporating **P3**'s pyrazine A ring and modifications of its B ring, in addition to the 2-azetidinone scaffold. Analogues illustrated in Figure 8.13 were considered as potential **P3** β -lactam leads. Analogue **145** was designed as a hybrid analogue to mimic the CA-4's B ring 4-methoxy while replacing the 3,4,5-trimethoxyphenyl A ring of CA-4 with **P3**'s pyrazine A ring. Analogues **141**, **142** and **143** were the most desirable for synthetic isolation, containing the B ring 2-hydroxyl substitution analogous to parent *trans* **P3** (Figure 8.13). This moiety would require OTBDMS protection of aldehyde precursors in preparation for reaction in Reformatsky or Staudinger reactions.

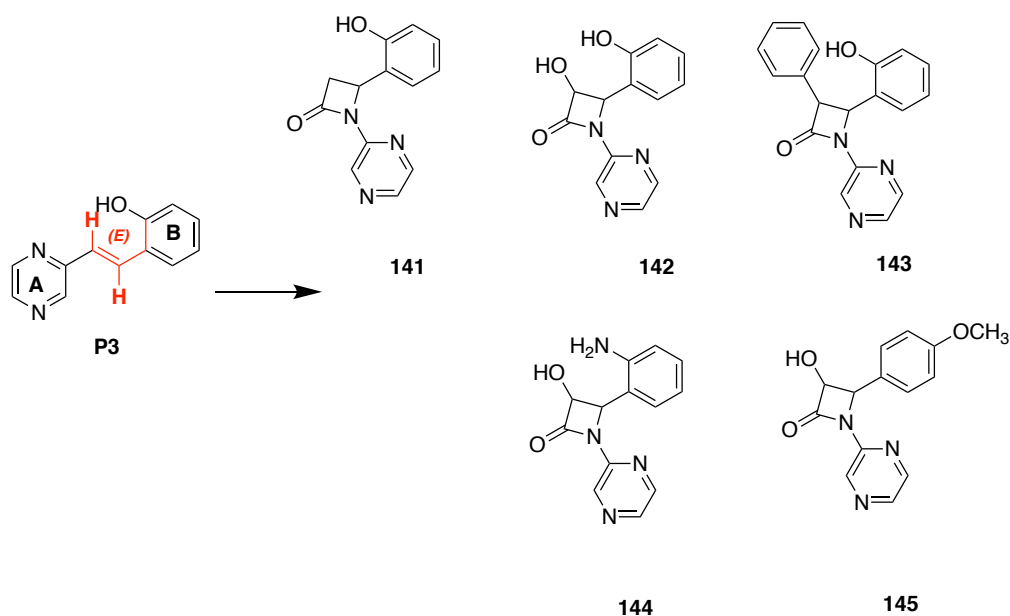
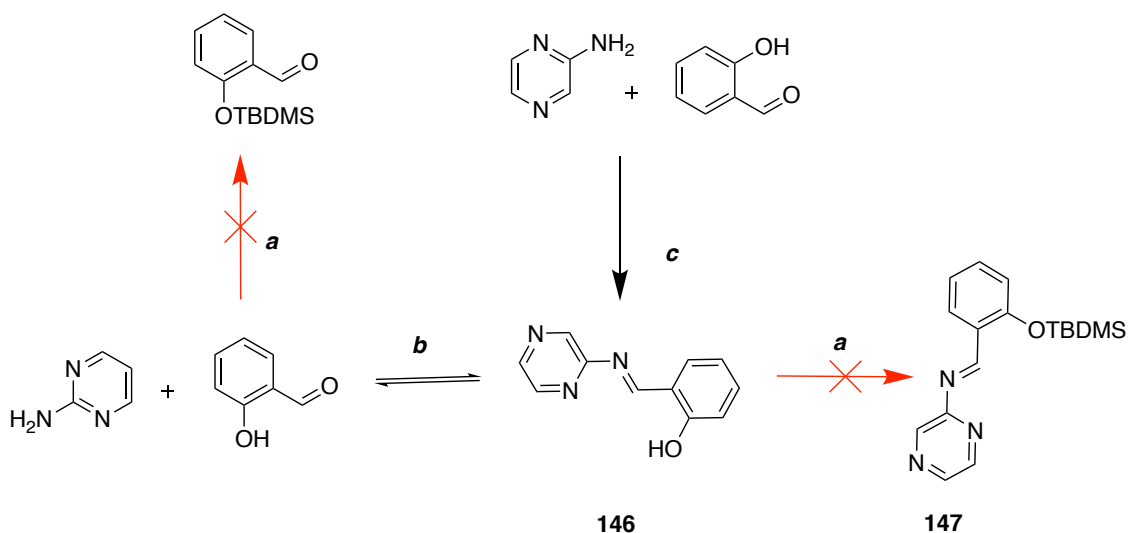
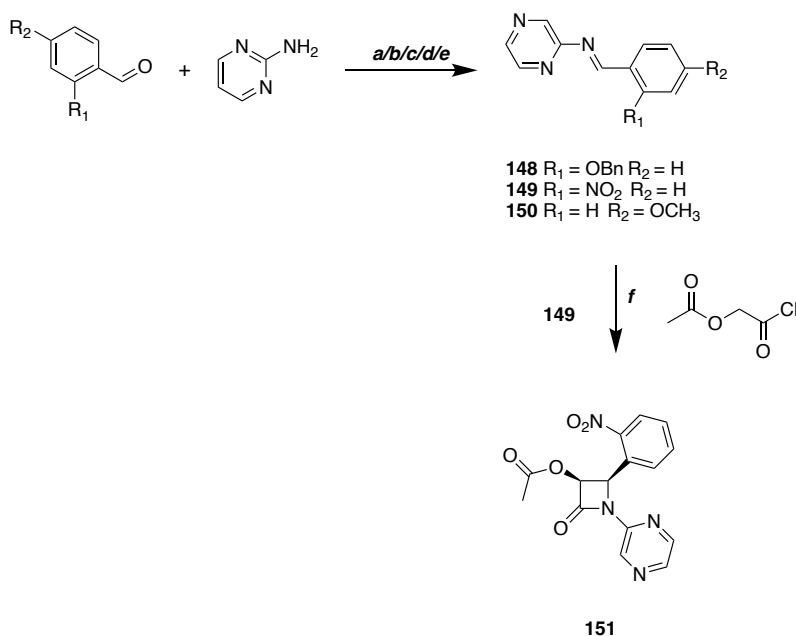


Figure 8.13: Hypothetical lead analogues of *cis* restricted **P3** β -lactam racemates

Two synthetic approaches were considered for TBDMS protection of the B ring hydroxyl moiety, (**a** and **b**, Scheme 8.6) Partial TBDMS protection of precursor salicaldehyde was observed on ^1H NMR (**a**, Scheme 8.6). However progressive degradation occurred over time, observed as two aldehyde protons at δ 10.4 ppm for TBDMS protected salicaldehyde and δ 9.9 ppm for parent salicaldehyde on ^1H NMR. Direct protection of imine **146** [synthesised in high yields using conventional reflux, (**c**, Scheme 8.6)] to yield the required imine (**147**) was also unsuccessful and resulted in imine degradation towards starting material (**b**, Scheme 8.6).



Scheme 8.6: Synthetic routes for synthesis of OTBDMS protected imine **147**. **a**: TBDMSCl, DBU, anhydrous DCM, N₂, RT **b**: breakdown of **146** to 2-aminopyrazine and salicylaldehyde **c**: Condensation of 2-aminopyrazine and salicylaldehyde in ethanol 90 °C

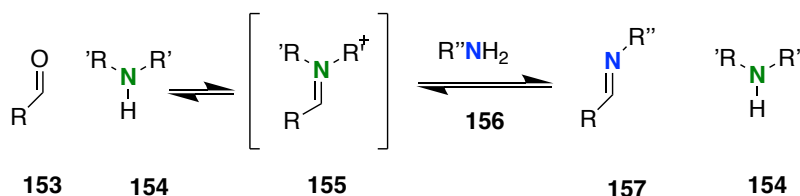


Scheme 8.7: Synthetic routes explored to obtain imines **148-150** and subsequent synthesis of β -lactam **151** **a**: Dean Stark apparatus in anhydrous toluene. **b**: Dean Stark apparatus with anhydrous toluene (30 mL) with ethanol as co-solvent. **c**: TiCl₄, *n*-butylamine, toluene, 0 °C, then RT overnight. **d**: aminocatalysis: Pyrrolidine (20 mol %), EtOH, 7h, reflux **e**: microwave irradiation: 2-aminopyrazine, substituted aldehyde, 60 minutes. Standard powder (CEM microwave synthesiser) **f**: TEA, toluene, reflux, 5h

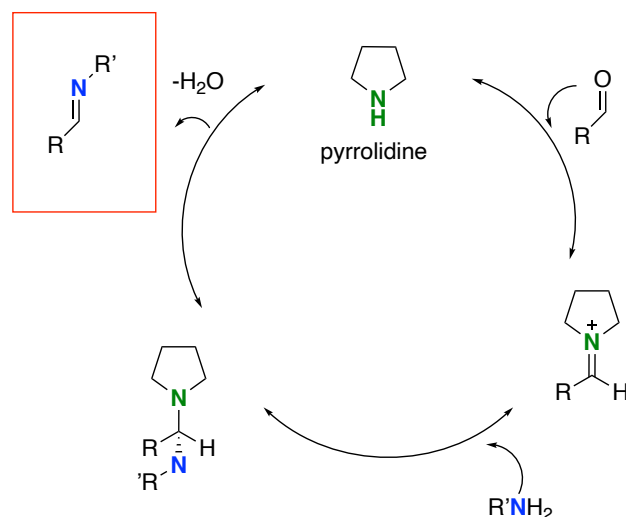
Subsequently attempts at condensing the commercially available benzyl ether protected aldehyde to yield imine **148** was explored using a plethora of trial reactions, including reflux in anhydrous toluene using Dean Stark apparatus (**a**, Scheme 8.7), reflux in anhydrous toluene with ethanol added as a co-solvent to solubilise 2-aminopyrazine also using the Dean Stark apparatus (**b**, Scheme 8.7), the use of TiCl₄ in presence of *n*-butylamine as base (**c**, Scheme 8.7), an amino catalytic approach with *n*-pyrrolidine as the catalyst (**d**, Scheme 8.7) and finally the use of the CEM microwave reactor with hot ethanol (**e**, Scheme 8.7). **148** was not observed or isolated demonstrating that benzyl ether protection of salicaldehyde's 2-hydroxyl electron donating moiety, prevents the aldehyde condensing with 2-aminopyrazine. Attempts to initiate the reaction using acid catalysis also failed, instead protonating the 2-amino pyrazine's primary amine to the unreactive salt.

Synthesis of imines using aminocatalytic strategies was also explored. (**d**, Scheme 8.7) Compounds containing NH₂ moieties (**154**, Scheme 8.8) may behave as nitrogenated nucleophiles permitting formation of C=N bonds (**157**, Scheme 8.8) *via* an iminium organocatalyst intermediate (**155**, Scheme 8.8).⁷⁶⁸ The electrophilicity of the iminium intermediate (**155**, Scheme 8.8) is more than 10 times greater than the carbonyl group in the precursor aldehyde (**153**, Scheme 8.8).⁷⁶⁹ This pathway is suggested to proceed in presence of weakly nucleophilic primary amines which ordinarily are inert in presence of aldehydes. Pyrrolidine has successfully demonstrated *N*-sulfinyl imine formation and was therefore considered as a synthetic strategy for potential access to **P3** imines (**148-150**, Scheme 8.7) using 2-aminopyrazine and the relevant aldehyde.⁷⁶⁸ A proposed mechanism is outlined in **B**, Scheme 8.8. The relevant aldehyde and 2-aminopyrazine were added to ethanol containing 20 mol % of pyrrolidine as the nucleophilic catalyst followed by reflux conditions for 7 hours (**d**, Scheme 8.7). The amino catalytic method was employed for synthesis of precursor imines **149** and **150** but was unsuccessful for the synthesis of benzyl ether protected **148**.

A

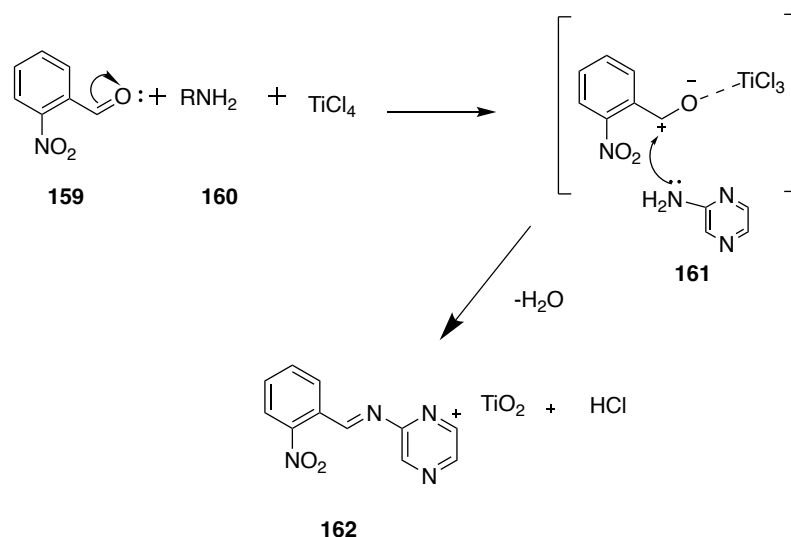


B



Scheme 8.8: **A:** Amino catalytic organocatalysed functionalisation of aldehydes using iminium activation with nitrogen based nucleophiles to form imines. **B:** Mechanism of pyrrolidine mediated amino catalytic imine formation through the iminium intermediate.⁷⁶⁸

Titanium tetrachloride (TiCl₄) has also demonstrated efficacy for imine synthesis by employing excesses of both amine and TiCl₄. Scheme 8.9 details the mechanism of TiCl₄ imine synthesis involving coordination of the carbonyl oxygen of the aldehyde (159, Scheme 8.9) with the titanium atom of TiCl₄ which activates the carbonyl for reaction to form intermediate **161** (Scheme 8.9). The intermediate (**161**) reacts with the primary amine (**160**),³⁴² followed by transfer of the carbonyl oxygen to the titanium atom, yielding the desired imine (**162**) with titanium dioxide (TiO₂) as a by-product.⁷⁷⁰ 2-Nitrobenzaldehyde was successfully reacted with 2-aminopyrazine, albeit in poor yield, using the TiCl₄ approach for imine synthesis (Scheme 8.9). **149** was confirmed by HRMS with target mass of 229.0719 [APCI (M+H⁺)]. **P3** imine reactions did not reach completion and due to issues with purification, imines were employed in Staudinger or Reformatsky reactions without further purification.



Scheme 8.9: Synthesis of imines *via* a titanium-aldehyde co-ordination complex using TiCl_4 . Reaction conditions: TiCl_3 , anhydrous toluene at 0 °C, 2-aminopyrazine, 10 minutes, then *n*-butylamine. RT overnight.

The synthesis of *cis* restricted **P3** β -lactam analogues was explored using **149** and **150** in Reformatsky and Staudinger reactions. Reformatsky reactions did not yield β -lactam products using either **149** or **150**. **151** (Figure 8.14 and Figure 8.15) was the only β -lactam racemate isolated as a *cis* restricted **P3** Pyrazet, synthesised using acetoxyacetyl chloride and the Staudinger reaction. Isolated yields were extremely poor (< 26%), with the lowest yield obtained using the TiCl_4 approach for synthesis of **149** followed by reaction with acetoxyacetyl chloride [1.21%, (**c**, Scheme 8.7)]. Synthesising the required imine using the Dean Stark apparatus with ethanol as co-solvent resulted in a final yield of 25% (**b**, Scheme 8.7) while the amino catalytic approach resulted in an isolated yield of 26% yield (**d**, Scheme 8.7) for **151** in the Staudinger reaction. The ^1H NMR spectrum of the Staudinger product **151** demonstrated predominantly *cis* isomer (at positions 3 and 4 of the β -lactam ring) in 82% yield (Figure 8.14). XRD analysis confirmed the *cis* configuration for **151** with chiralities confirmed as 3*S*,4*R* and 3*R*,4*S* for enantiomers of racemate **151** (Figure 8.15). Further reaction to reduce the B ring's 2- NO_2 substitution of **151** to the 2- NH_2 moiety did not yield the desired product. Similarly hydrazinolysis of the 3-acetoxy using hydrazine dihydrochloride did not afford the final 3-hydroxyl product. The desired final Pyrazet, **144** (Figure 8.13) was therefore not isolated in this thesis.

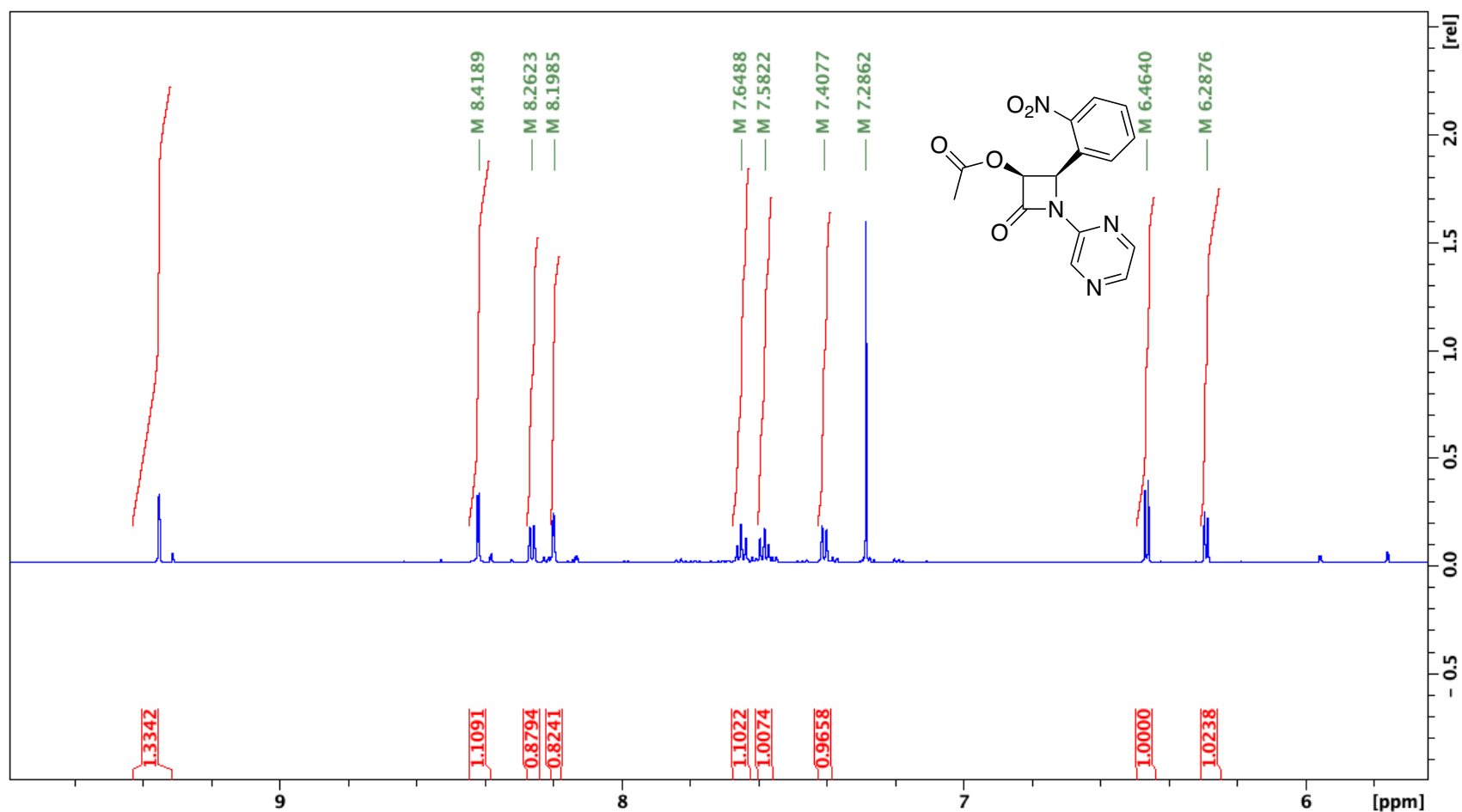


Figure 8.14: ^1H NMR of **151** in CDCl_3 at 600 MHz illustrating majority presence of *cis* isomer (doublets with $^3J = 5$ Hz at δ 6.46 and 6.29 ppm) and presence of *trans* isomer (doublets at δ 5.96 and 5.76 ppm with ^3J values of 2 Hz).

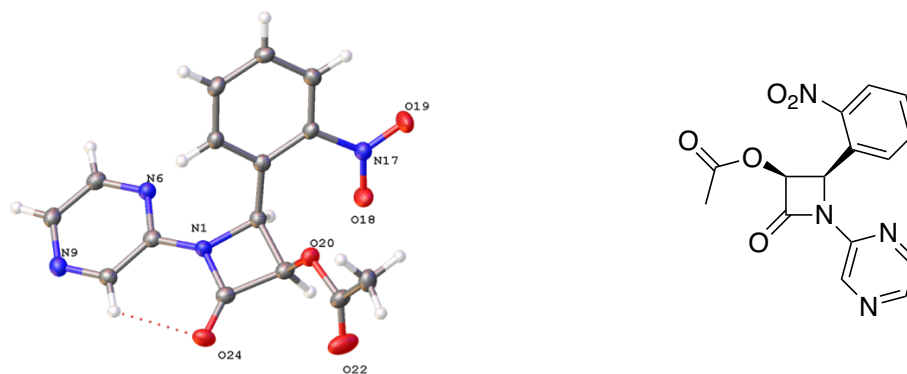


Figure 8.15: Molecular structure of *cis* **151** with atomic displacement shown at 50% probability. Heteroatoms labelled only for clarity. Hydrogen bonding indicated by dotted lines. One enantiomer illustrated.

8.14 Anti-proliferative evaluation of **151** by National Cancer Institute

The National Cancer Institute (NCI) operates a developmental therapeutics program including the NCI-60 tumour cell line screen for over 20 years. It uses 60 tumour cell lines to identify novel compounds as anti-cancer agents and can distinguish between both growth inhibition and cell death in tumour cells. The 60 cell lines encompass 9 different cancer subtypes representing leukaemia, melanoma, lung, colon, brain, ovarian, breast, prostate and kidney cancers.⁷⁷¹ The NCI screen uses a method of protein concentration detection to determine % viabilities in treated cells using a dye called sulforhodamine B (SRB) solution which differs from AlamarBlue™ previously described in this thesis.

Compounds submitted to the NCI undergo a preliminary screen in a one dose assay at a concentration of 10 μ M. If the pre-determined threshold for inhibition is met, compounds progress to a 5-dose assay. The NCI-60 screen is a five dose screen that reports three parameters across 60 cell lines (Table 8.4).⁷⁷²

- 1) GI₅₀ detailing drug concentration resulting in 50% total cell growth inhibition, less commonly referred to as the absolute IC₅₀.⁶⁹⁸ This compares to relative IC₅₀ values described in Chapter 6 which report the concentration of a drug required to reduce population of cells growth response to 50% with respect to vehicle control.⁷⁷³
- 2) TGI considered as the concentration required for total growth inhibition
- 3) LC₅₀ considered as the concentration required to induce 50% lethality (measurement of cytotoxic rather than antiproliferative effect in cancer cells).

In the one-dose screen, the mean growth % of all 60 cell lines in initial one dose screening for **151** was 17%. Parent **P3** was also screened at 10 μM in the NCI-60 panel and was not active as an antiproliferative agent with a mean % growth value of 99%. This data demonstrates that insertion of the β -lactam ring to *cis* restrict **P3**'s A and B rings in addition to substitution of the B ring 2-hydroxyl with a 2-nitro moiety dramatically augments the antiproliferative effects of **P3**. **P3P** has been further selected for 5 dose screening which has not yet progressed.

GI_{50} values from the five-dose screen are presented for **151** in Table 8.4. For most cell lines, GI_{50} values were determined below 1 μM . In the breast cancer cell panel of interest, **151** demonstrated moderate to good biological activity. GI_{50} values were determined as 0.32 μM , 0.41 μM , 0.74 μM and 0.32 μM in MCF-7, MDA-MB-231, Hs578T and BT-549 cells respectively (Table 8.4 and **A**, Figure 8.16). This antiproliferative activity did not extend to HT-29 cells which had a much higher GI_{50} value of 2.84 μM (Table 8.4 and **B**, Figure 8.16). **151** was determined as most active in the non-small-cell-lung cancer cell line NCI-H226 (GI_{50} of 0.257 μM), CNS cancer cell lines SF539 (GI_{50} of 0.244 μM) and SNB-75 (GI_{50} of 0.21 μM) in addition to two melanoma cell lines; LOX-IMV1 (GI_{50} of 0.241 μM) and MDA-MB-435 [GI_{50} of 0.196 μM , (Table 8.4)].

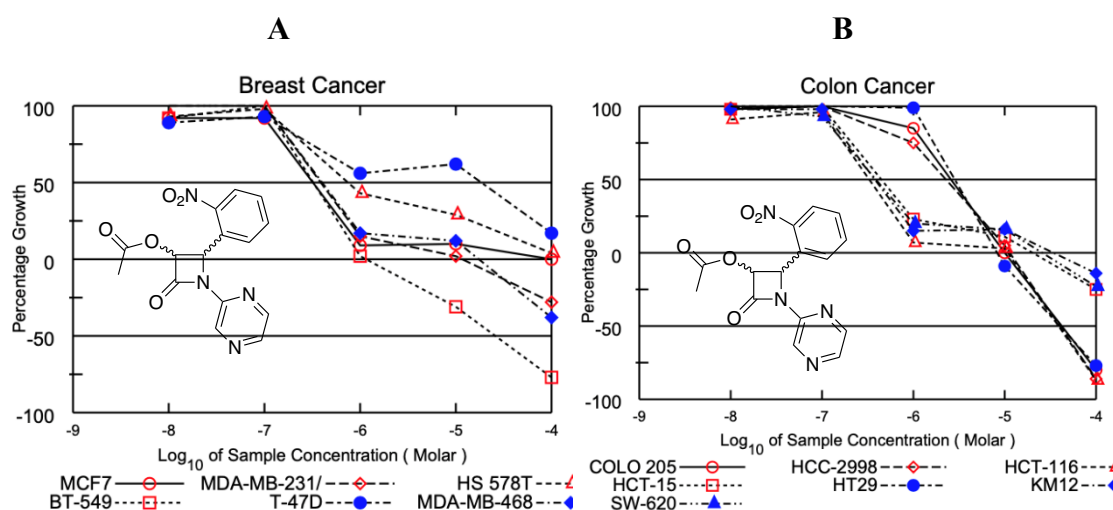


Figure 8.16: Dose-response curves generated by NCI-60 screening for **151** for **A**: breast cancer cell lines **B**: colon cancer cell lines.

Table 8.4: GI_{50} values for **151** in NCI 5-dose screen

Cell Line	GI ₅₀ (μM)	Cell Line	GI ₅₀ (μM)
Leukemia		Melanoma	
CCRF-CEM	0.452	LOX IMV1	0.241
HL-60 ⁷⁷⁴	0.336	MALME-3M	0.755
K-562	0.337	M14	0.315
MOLT-4	0.475	MDA-MB-435	0.196
RPMI-8226	0.371	SK-MEL-2	1.96
SR	0.344	SK-MEL-28	1.12
Non-Small Cell Lung Cancer		SK-MEL-5	0.49
A549/ATCC	0.409	UACC-257	0.705
HOP-62	0.594	UACC-62	0.357
HOP-92	0.692	Ovarian Cancer	
NCI-H226	0.257	IGROV1	0.531
NCI-H23	0.32	OVCAR-3	0.357
NCI-H332M	6.63	OVCAR-4	0.863
NCI-H460	3.51	OVCAR-5	0.746
NCI-H552	1.98	OVCAR-8	0.321
NCI/ADR-RES	0.387	SK-OV-3	0.618
Colon Cancer		Renal Cancer	
COLO 205	2.58	786-0	0.684
HCT-2998	2.29	A498	>10e-4
HCT-116	0.326	ACHN	0.604
HCT-15	4.54	CAKI-1	0.37
HT29	2.84	RXF 393	0.271
KM12	3.76	SN12C	0.466
SW-620	3.89	TK-10	16.8
CNS Cancer		UO-31	0.663
SF-268	0.548	Breast Cancer	
SF295	1.24	MCF-7	0.32
SF539	0.244	MDA-MB-231/ATCC	0.407
SNB-19	2.18	HS 578T	0.737
SNB-75	0.21	BT-549	0.323
U251	0.372	T-47D	18.6
Prostate cancer		MDA-MB-468	0.422
PC-3	0.603		
DU-145	0.512		



Red = good activity, yellow = moderate activity and green = poor activity

Table 8.5 presents the TGI and LC₅₀ values in breast cancer cells for **151**. Both values are greater than the GI₅₀ values for 50% cell inhibition indicating that the dose required for total inhibition and lethality of cancer cells is much higher than the concentration required for 50% inhibition, inferring potential for semi selective toxicity towards cells at treatment doses.

Table 8.5: GI₅₀, TDI₅₀ and LC₅₀ values for **P3BL1** in breast cancer cell lines from NCI-60 5 dose screen

Breast cancer cell line	GI ₅₀ (μM)	TGI (μM)	LC ₅₀ (μM)
MCF-7	0.32	>100	>100
MDA-MB-231/ATCC	0.407	11.9	>100
HS 578T	0.737	>100	>100
BT-549	0.323	1.16	25.8
T-47D	18.6	>100	>100
MDA-MB-468	0.422	17.5	>100

These findings demonstrate the potential of the pyrazine β-lactam scaffold as a potential antiproliferative agent in contrast to the *trans* **P3** which did not demonstrate any antiproliferative activity (Figure 8.9). The β-lactam pyrazets have potential to supersede **P3** as an AA and radiosensitising agent with additional antiproliferative properties. Future work must involve determination of whether insertion of the β-lactam ring structure maintains the radiosensitising effects of **P3** in addition to augmenting its antiproliferative effects. Microtubule depolymerisation potential for **151** remains to be elucidated. A further panel of β-lactam pyrazets should also be explored *i.e* reduction of the NO₂ substitution of **151** to NH₂, hydrazinolysis or methanolysis of **151**'s OCOCH₃ to the 3-hydroxyl and OTBDMS or other protection of the B ring 2-hydroxyl. Additionally, 3-hydroxyl substituted pyrazet racemates are candidates for further progression toward their enantiomers as described in resolution approaches in Chapters 3,4 and 7.

8.15 Chapter summary and outlook

P3 is highly insoluble and only isolated to date as the *trans* isomer. Solubility issues have been overcome for **P3** using the phosphoric acid derivative **P3P**. **P3P** has a better antiproliferative profile than parent **P3** in breast cancer cell lines with preliminary cell cycle data (n=1) demonstrating potential anti-mitotic activity (G₂/M phase arrest) and

apoptosis. Additionally, it is rapidly hydrolysed to **P3** by AP enzymes demonstrating its viability for use as the phosphate prodrug, rapidly converted into parent **P3** *in vivo*. Computational studies for *cis* and *trans* **P3** at the CBS have that demonstrated that *cis* **P3** may have microtubule depolymerising activity as a CBSI while *trans* **P3** may not. A pyrazine β -lactam pyrazet analogue **151**, was synthesised and its antiproliferative activity evaluated in the NCI-60 5 dose cell line screen. NCI data demonstrates superior antiproliferative activity in the panel of cancer cells lines for **151** compared to parent **P3**. Future work must involve optimisation of the pyrazine β -lactam scaffold for maximum biological activity. Synthetic or photochemical strategies for isolation of *cis* **P3** should also be explored. The microtubule depolymerising effects of *cis* **P3** and the panel of pyrazets should be evaluated. Additionally, further optimisation for 3-substituted pyrazine β -lactams should involve synthesis of their *trans* derivatives and isolation of their enantiopure forms for maximum anti-proliferative activity.

Chapter 9 - Concluding remarks and future work

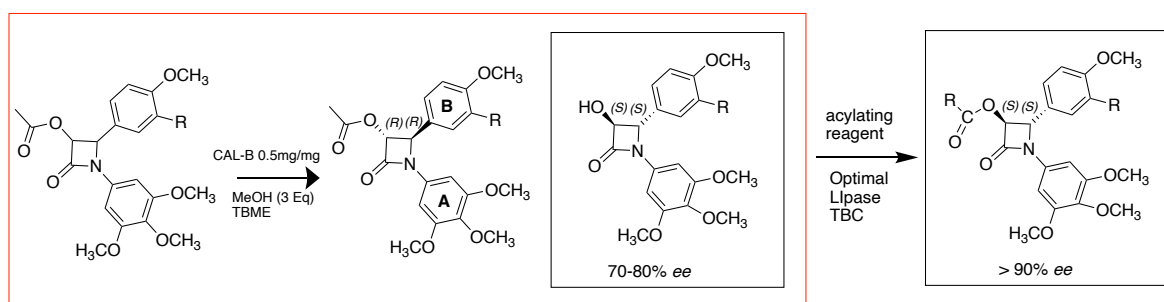
Despite advances in treatment approaches for both TNBC and metastatic colorectal cancer, limitations associated with managing recurrent and advanced disease remain.¹⁵⁶ At present, chemotherapy such as taxanes and anthracyclines are the predominant treatments for patients with TNBC.¹¹³ Adjuvant therapy is often ineffective for metastatic TNBC with median survival estimated at only 13 months.^{114, 775} Receptor tyrosine kinases inhibitors such as EGFR inhibitors are now in development as novel agents for the treatment of resistant cancers. Lapatinib is the only anti-EGFR agent approved by the FDA for clinical use, and it is recommended to use in combination with chemotherapy or hormone therapy.⁷⁷⁶ Since over 50% of patients with TNBC demonstrate overexpression of EGFR, anti-EGFR was anticipated as a promising target for TNBCs. However clinical trials have consistently demonstrated poor effectiveness of anti-EGFR antibodies.^{777, 110, 778} Cyclin dependent kinases (CDKs) 4/6 which participate in cell cycle progression from G1 to DNA synthesis (S phase) mediate tumorigenesis.^{779, 780} Three CDK inhibitors have now been approved for ER+ and HER2+ breast cancers *e.g* palbociclib,⁷⁸¹ robociclib⁷⁸² and abemaciclib.⁷⁸³ However no CDK4/6 inhibitor is currently approved for treating TNBC.^{784, 785} Trilaciclib is in trials for patients receiving first or second line treatment for mTNBC. In summary, strategies for the treatment of mTNBC demand novel approaches for combination regimens when other agents fail.⁷⁸⁶ Notwithstanding limitations associated with treatment of TNBC, mCRCs are commonly resistant to first line and commonly used clinical agents. Additionally CA-4 has no effect as an anti-proliferative, microtubule depolymerising or anti-mitotic agent in colorectal cancer cells.⁷⁸⁷

Racemates of the combretazet panel discussed in this thesis have previously been reported with excellent *in vitro* activity in both breast and colorectal cancer cell lines.^{357, 359, 378, 400} This thesis has reported methods for enantioseparation of combretazet racemates ranging from 50% to >90% *ee*. Comparative *in vitro* biochemical analysis has demonstrated the advantages of eutomers of enantiomeric pairs. Eutomers demonstrated dramatically augmented anti-proliferative, microtubule depolymerising and anti-mitotic activity with respect to both the corresponding parent racemates and distomers. Preliminary pre-clinical *in vitro* analysis discussed in this thesis demonstrates the potential for treatment of advanced mTNBC and/or mCRC with B ring *meta* hydroxyl substituted combretazet eutomers.

This project has demonstrated the limitations associated with use of chiral diastereomeric resolution as a method for enantiopure isolation of the combretazet eutomers. Co-elution of diastereomers during LC consistently reduced the yield of the desired eutomers in addition to reducing *ee* of the pre-distomer diastereomer fractions. While isolated yields in this thesis were sufficient for progression toward preliminary *in vitro* analysis, larger yields will be required in future for progression towards *in vivo* studies. The water solubility of parent eutomers remains to be elucidated. Based on prior reports for the parent racemates³⁸⁰ it is anticipated that progression toward pre-clinical *in vivo* models will mandate derivatisation toward the phosphate or phosphate-like prodrugs, such as the novel anti-viral remdesivir as a proline phosphoroamidate prodrug used for the treatment of COVID-19.^{317, 788} Our group have previously demonstrated that β -lactam racemate derivatives of CA-4 are below the detectable limit of solubility measured using HPLC. These solubility issues which would otherwise limit the pre-clinical progression of combretazet eutomers have previously been overcome for the racemic forms by derivatisation toward phosphate or amino acid prodrugs.³⁸⁰ Our panel of 3-hydroxyl analogues containing a variety of substitutions and deletions at the B ring *meta* hydroxyl have dual anti-cancer activity in both TNBC cells and in chemo resistant HT-29 colorectal cancer cells.^{378, 400} They have synthetic potential for development as the phosphate prodrugs due to the availability of the hydroxyl functional group for phosphate ester derivatisation. However, the chemistry required for prodrug synthesis including, synthetic optimisation, solubility studies and subsequent biological evaluation requires larger enantiopure isolated yields than chiral resolution has provided in this thesis. For this reason, KR was explored as a second and alternative method for enantiopure β -lactam isolation in Chapter 7. KR demonstrated improved isolated yields in a more accessible, sustainable and facile approach for enantioseparation. Future adoption of KR may enable synthesis of a panel of prodrug derivatives of the 3*S*, 4*S* eutomers for further pre-clinical evaluation. *Ee* values for eutomers isolated in this thesis using KR remain sub-optimal ranging only from 60-80% *ee*.

Initial results using KR demonstrated poor enantioselectivity using 1 mg/mg of CAL-B per 3-acetoxy β -lactam in combination with 10 equivalents of methanol. 3-Hydroxyl 3*S*,4*S* **17EN1** was isolated in 56% *ee*. The optimised method demonstrated that by reducing the rate of CAL-B mediated methanolysis using 0.5 mg/mg of CAL-B per mg

of 3-acetoxy β -lactam with 3 equivalents of methanol (in TMBE), *ee* increased from 40-50% to 70-80% could be obtained. There is currently scope to further augment *ee* values using a more optimal lipase enzyme or an alternative synthetic route *e.g* employing lipase enzymes to acylate the 3-hydroxyl moiety following initial methanolysis as a component of a double resolution approach outlined in Scheme 9.1. Double resolution techniques for gram scale preparation of β -lactam enantiomers are previously reported, involving acylation with an acyl donor to yield 88% *ee* followed by alcoholysis to an *ee* value of >99% using *Pseudomonas cepasia* (lipase PS).⁷²⁸ Specifically, long terms aims are to apply the most optimal approach using lipase enzymes as biocatalysts to obtain enantioenriched, but ideally enantiopure 3*S*,4*S* eutomers, followed by synthesis of the most clinically viable amino acid and/or phosphate prodrug. Future visions for the combretazets include progression of a clinically viable B ring *meta* substituted prodrug *e.g* the phosphate prodrug of **18EN1** towards pre-clinical and clinical *in vivo* models, as novel agents with dual activity for the treatment of both TNBC and chemo resistant colorectal cancers.



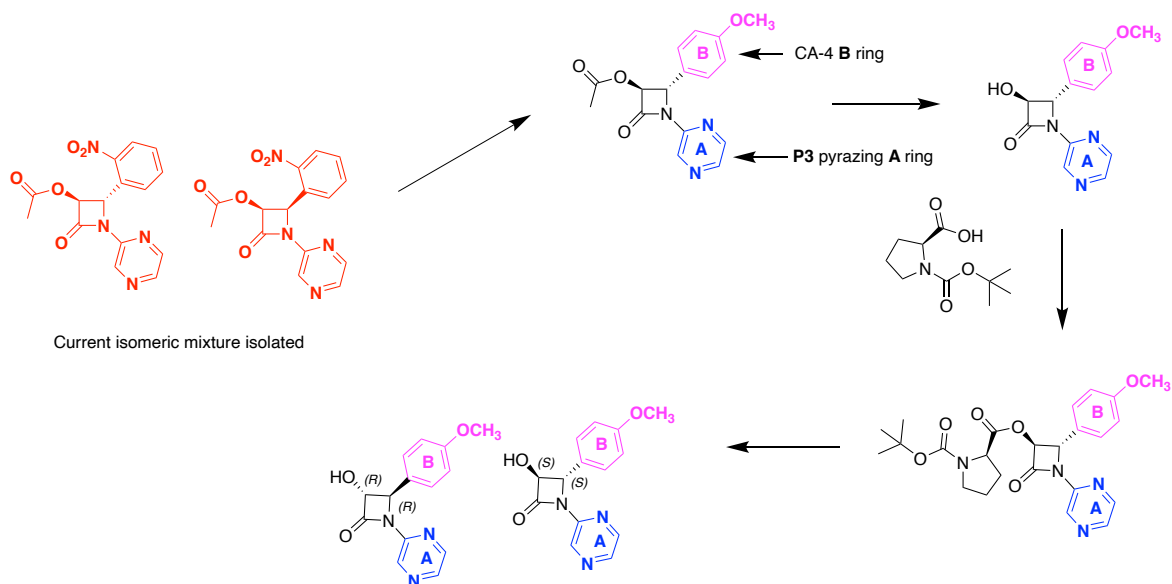
Scheme 9.1: Proposed biocatalytic pathway for achieving > 99% *ee* for 3*S*,4*S* eutomers of 3-hydroxyl β -lactam racemates. Pathway carried out and *ee* values determined to date highlighted in red. TBC = to be confirmed.

Recent years have catalysed interest in more environmentally friendly strategies for synthetic chemistry, particularly in the area of green technology development.^{789, 790} The use of mechanochemical activation of immobilised lipase enzymes in a field known as mechanochemistry or mechanoenzymology^{791, 792} has seen application of KR for asymmetric induction to yield chiral compounds and has led to the synthesis of a wide variety of chiral APIs, *e.g* precursors of one of the most frequently used β -blockers propranolol.^{791, 793} CAL-B specifically, has demonstrated excellent KR of several APIs and precursors by means of mechanoenzymology in enantioselective acylation and/or

hydrolytic procedures.^{750, 794, 795} Good results have been achieved for such propranolol precursors *via* enantioselective esterification in stainless steel, agate or Teflon milling jars accompanied by vibrational milling at 25 Hz in 90% *ee* with an *E* value of 21.⁴⁴² Hydrolytic KR was also explored using water instead of an acylating reagent producing 96% *ee* and selective 50% conversion with impressive *E* values of 259, using agate milling jars. Mechanoenzymatic reactions are typically facilitated by liquid assisted grinding (LAG), crucial for driving the reaction forward⁷⁹⁶ by ensuring homogenous distribution of immobilized catalyst and substrate.⁷⁹⁷ Best results were obtained using dioxane as the LAG additive, agate milling and CAL-B in a ratio of 1:1 with substrate, with *ee* of >99%, *E* value of 300 and conversion ceasing at 50%.⁴⁴²

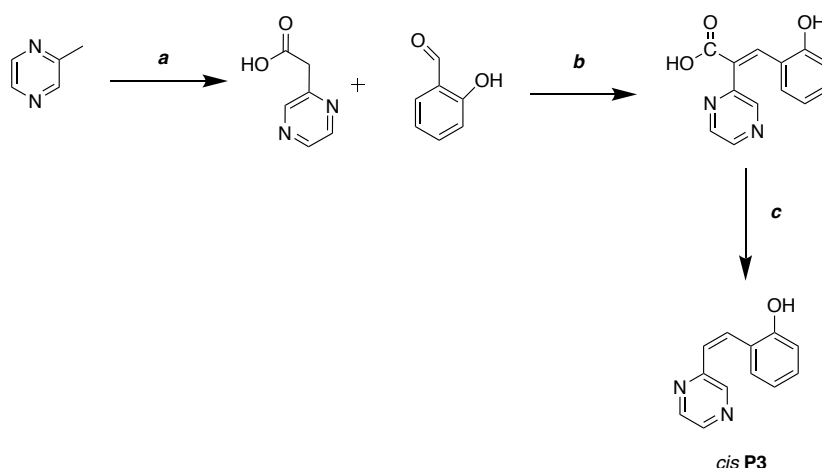
While CAL-B typically is considered as an enzyme which does not demonstrate interfacial activation, it is now suggested that bulkier substrates gain better access to active site due to conformational change of the $\alpha 5$ or $\alpha 10$ helix at the enzyme's lid.⁷⁴⁵ Mechanical force has been demonstrated as altering CAL-B activity most likely due to alteration of $\alpha 10$ helix conformation.⁷⁹⁸ This provides strong rationale for further investigation of enantiopure combretazet isolation using mechanoenzymology in future studies.

Additionally, in this thesis, expansion of the 3,4,5-trimethoxyphenyl structure for anti-proliferative activity has been explored by incorporating the pyrazine A ring for **P3** β -lactam analogues as a method of *cis* restriction for AA agent **P3** (Chapter 8) This work now requires full elucidation of the mechanisms of anti-proliferative activity including tubulin polymerisation analysis and cell cycle studies. This thesis has demonstrated that the presence of the B ring *para*-methoxy substituent is essential for anti-proliferative activity due to the dramatic reduction in antiproliferative activity observed for B ring *para* fluorine and nitro substituted analogues **89-91** with respect to *e.g* **17** (Chapter 6). Formation of CA-4/**P3** hybrids as illustrated in Scheme 9.2 with the pyrazine A ring, *para* methoxy substituted B ring and 3-hydroxyl, would enable chiral resolution and isolation of the respective enantiomeric forms (Scheme 9.2). Enantioseparation in turn has the potential for augmentation of bioactivity due to presence of the desirable substitution pattern.



Scheme 9.2: Proposed CA-4-P3 β -lactam hybrid with potential strategies for chiral resolution

Synthesis of *cis* P3 should also be explored to determine its microtubule depolymerising activity and CBS affinity. A proposed method is detailed below, involving carboxylation of 2-methylpyrazine towards its corresponding acetic acid for Perkin condensation with salicylaldehyde towards the carboxylic acid derivative, which may then be decarboxylated to the *cis* stilbene structure (Scheme 9.3). This method has previously been reported for the synthesis of *cis* CA-4 and its derivatives.³⁴³



Scheme 9.3: Proposed Perkin condensation synthesis for *cis* P3: **a**: *n*-butyl lithium, DIPEA, THF, -78 °C, quench with solid CO₂. **b**: acetic anhydride, TEA, 120 °C, CEM microwave synthesiser, 30 minutes. **c**: Decarboxylation with copper powder, quinoline, 200 °C, 3h

An alternative to the synthetic isolation of *cis* **P3** is the potential for a photoisomerisation reaction which promotes a switch from isolated *trans* or (*E*) configuration to the corresponding *cis* (*Z*) isomer. Photoisomerisation in opposite *trans* to *cis* configuration is a counter thermodynamic process and not commonly found in the literature. However examples are described for sterically hindered *cis* isomers using solar irradiation, a green way to carry out organic synthesis⁷⁹⁹ and is therefore worthy of further investigation.

Both *cis* **P3** and a potential panel of *cis* restricted **P3** β -lactam racemates and/or enantiomers have scope as potential CBSIs, VDAs and AA agents with more potent anti-proliferative and radiosensitising activity with respect to parent *trans* **P3**, each of which remain to be investigated.

The underlying mechanism for anti-mitotic activity observed for **P3P** also requires confirmation and further investigation. The mechanisms for the large improvement in anti-proliferative activity for the phosphate prodrug versus parent *trans* stilbene structure requires extensive elaboration. Potential explanations include increased cell uptake either by active or passive diffusion.

To summarise, both the combretazets and **P3** analogues have demonstrated excellent potential for the treatment of TNBC and colorectal cancers as CBSIs, VDAs, AAs and radiosensitising agents. Synthetic optimisation is required for both series prior to progression toward future pre-clinical *in vivo* stages. Nevertheless, these agents offer potential strategies for future curative and effective management of resistant, advanced and/or metastatic breast and colorectal cancers, and are therefore worthy of further development.

Chapter 10 - Experimental

Materials and Methods

All reagents were commercially available and were used without any further purification unless otherwise indicated. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR Paragon 1000 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded at 25 °C on a Bruker Avance III 400 or Avance II 600 (400.13 MHz/ 600.13 MHz, ^1H ; 100.61 MHz, ^{13}C /150.61 MHz, ^{13}C) in either CDCl_3 or $\text{DMSO}-d_6$. For CDCl_3 , the internal standard is tetramethylsilane (TMS) with ^1H - NMR spectra assigned relative to this TMS peak at δ 0.00 ppm. ^{13}C -NMR spectra were assigned relative to the middle peak of CDCl_3 triplet at δ 77.00 ppm. For ^1H NMR assignments, chemical shifts are reported: shift value (multiplicity, integral value, coupling constant(s)). For ^1H -NMR assignments, chemical shifts are reported: shift value (multiplicity, the integral value representing number of protons, coupling constant(s), relevant proton or carbon assignment, isomer, diastereomer and or rotamer composition). Quantitative (q) ^1H experiments utilised a 90° excitation pulse and a 60 second recycle delay between scans to allow the spins to return to equilibrium ensuring 5 times the T_1 (spin lattice relaxation) was exceeded. High resolution mass spectrometry (HRMS) was carried out by Dr. Gary Hessman of the School of Chemistry, Trinity College Dublin. Experiments were carried out predominantly using atmospheric pressure chemical ionisation (APCI) with occasional use of electrospray ionisation (ESI-MS) in both positive and negative modes. ESI mass spectra were acquired using a Bruker micrOTOF-Q III spectrometer interfaced to a Dionex UltiMate 3000 LC in positive and negative modes as required. Masses were recorded over the range 100-1400 m/z . APCI experiments were carried out on a Bruker micrOTOF-Q III spectrometer interfaced to a Dionex UltiMate 3000 LC or direct insertion probe. The instrument was operated in positive or negative mode as required. Masses were recorded over a range of 100-1600 m/z . Low resolution mass spectrometry (LRMS) was performed by Mr Brian Talbot of the School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin using APCI on the LTQ Orbitrap Discovery Mass Spectrometer (ThermoFisher Scientific), operating in positive/negative ion polarity switching mode at mass range of 50 to 2000 m/z . TLC was carried out on silica gel on Merck silica gel on TLC aluminium foils with fluorescent indicator F-254 nm. R_f values are quoted for each compound. Flash column chromatography was carried out on Merck silica gel technical grade, pore size 60 Å, 40-63 μM particle size, 230-400 mesh. Microwave experiments were carried out using a Discover CEM microwave synthesizer on standard power setting unless otherwise stated. On standard power setting, the maximum power supplied is 300 W. X-ray crystallography data was collected by Dr. Brendan Twamley of the School of Chemistry, Trinity College Dublin.

Solubility & Analytical HPLC Studies

HPLC instrumentation consisted of a Waters 1525 Binary HPLC pump in-line vacuum degasser, a 717 plus auto sampler and a Waters 2487 dual wavelength detector. The column used was a BDS Hypersil C18, reverse phase, 250 x 4.6mm (ThermoFisher Scientific). 70:30 HPLC grade MeCN:H₂O as mobile phase, injection volume of 10 μ L and flow rate of 1 mL/min. A calibration of standard solutions versus concentration was plotted. All standard solutions were made from a 0.5 mg/ mL stock solution in mobile phase. Calibration curves were constructed in triplicate from three independent series of stock solutions made up on three independent occasions. A linear regression equation and correlation coefficient (R^2 value) were calculated in both Microsoft excel and GraphPad. An R^2 value of 0.98-0.99 was accepted. The shake flask method for solubility measurements was designed in line with WHO guidance. A suspension of relevant compound was weighed out and added with 1 mL HPLC grade water in a microcentrifuge tube or Eppendorf vial. This suspension was placed onto a mechanical shaker (Mini gyro- rocker, SSM3, Stuart) with a stirring speed of 70 rpm for 24 hours. After 24 hours the solution was filtered through a 0.22 μ M filter to remove undissolved compound. The filtrate was transferred to a HPLC vial and diluted to the linear range of relevant calibration curve with mobile phase prior to analysis by HPLC. Solubility studies were carried out in triplicate. Analytical stability studies were carried out at three pH values using the phosphate buffer recipes detailed in the British Pharmacopoeia as follows:

Phosphate buffer solution in pH 4.0: 1.7 g of potassium dihydrogen orthophosphate (KH₂PO₄) added to 175 mL of dH₂O and adjusted to pH 4 using 10 % v/v orthophosphoric acid. The solution is then made up to volume of 250 mL with dH₂O.

Phosphate buffer solution in pH 7.4: 4.08 g KH₂PO₄ added to 150 mL of dH₂O to make a 0.2M solution. Add 125 mL of above solution to 198 mL of 0.1 M NaOH solution.

Phosphate buffer solution in pH 9.0: 5.22g of KH₂PO₄ added to 240 mL dH₂O and pH adjusted to 9.0 by dropwise addition of 1M potassium hydroxide solution (5.6 g/ 100 mL of dH₂O). The final volume was diluted to 300 mL using dH₂O.

The test compound was dissolved in mobile phase (70:30 MeCN: H₂O) prior to dilution in phosphate buffer.

Chiral HPLC

Chiral HPLC was carried out for racemates and enantiomers using Chrompak-IH-3 (150 x 4.6 mm) supplied by Chiral Technologies Europe with a Chiral- IH-3 guard column, to protect the analytical column from impurities. The column utilises an immobilised chiral polysaccharide stationary phase, tris(*S*)- α -methylbenzylcarbamate. The following conditions were used for all chiral HPLC studies: injection volume 5 μ L, flow rate of 1 mg/mL and run time of 10-60 minutes, using n-hexane:propan-2-ol, 1:1 at 254 nm. All samples were dissolved in propan-2-ol or ethyl acetate at a concentration of 1-5 mg/ mL for optimal chromatograph resolution. *Ee* was calculated using the following formula: $(\% \text{ Major peak} - \% \text{ minor peak}) \times 100 = \% ee$. Racemates were analysed using a non-chiral reverse phase Zorbax SB-C18 column, 5 μ m particle size, 25 cm x 4.6 mm, with an injection volume of 10 μ L of sample, flow rate of 1mg/mL, 10 minute run time and mobile phase of 60:40% MeCN/H₂O.

Characterisation for of all novel analogues are detailed below while intermediates and previously reported analogues are reported in **Appendix 3**.

General Method I: Phenol protection using *t*-butyldimethylsilylchloride (TBDMSCl)

Route A: Direct protection of phenolic imines^{495, 504}

To a stirring solution of phenolic imine **46** (1 eq, 6 mmol, 1.9g) in anhydrous DCM (60 mL), *t*-butyldimethylsilylchloride (1.2 eq, 7.2 mmol, 1.08g) was added under a nitrogenous atmosphere at room temperature. DBU (1.6 eq, 9.6 mmol, 1.44 mL) was added dropwise to the reaction vessel and subsequently stirred for three hours until the starting material had disappeared, indicated by TLC. On completion the reaction mixture was diluted with dichloromethane (60 mL), washed with dH₂O (100 mL), 0.1 M HCl (50 mL) and a saturated solution of NaHCO₃ (50 mL), retaining the organic layer each time. The organic layer was dried with anhydrous Na₂SO₄, filtered and the solvent removed under reduced pressure. The resulting product was a brown oil as a mixture of parent aldehyde and amine with TBDMS protected imine.

Route B: Protection of precursor 3-hydroxy-4-methoxybenzaldehyde

To a solution of 3-hydroxy-4-methoxybenzaldehyde (1 eq, 10 mmol, 1.52g) in anhydrous DCM (30 mL) under nitrogen atmosphere at room temperature, *t*-butyldimethylsilylchloride (1.2 eq, 12 mmol, 1.81g) was added followed by the dropwise addition of DBU (1.8 eq, 18 mmol, 2.68 mL). The reaction was stirred at room temperature until completion as indicated by TLC (1-2 hours). On completion the reaction was diluted with 60-100 mL of DCM which was subsequently washed with dH₂O (100 mL), 0.1M HCl (50 mL) and NaHCO_{3(sat)} (50 mL) retaining the organic layer each time. The organic layer was dried with anhydrous Na₂SO₄,

filtered and the solvent removed under reduced pressure yielding **42** (**Appendix 3**) as a yellow oil for use without further purification in agreement with literature characterisation.

General Method II: Protection of amino phenylacetic acid with the CBZ protecting group⁴⁹⁵

Aminophenylacetic acid (1eq, 5mmol, 0.75g) and sodium bicarbonate (Na_2CO_3) (2.7 eq, 13.5 mmol, 1.13g) were dissolved in dH_2O (50 mL). The reaction vessel was placed on ice and cooled to 0 °C. Benzyl chloroformate (1.1 eq, 5.5 mmol, 0.78 mL) was dissolved in 4 mL of dioxane and injected *via* septum to the stirring solution. The reaction was stirred for 30 minutes at 0 °C before warming to room temperature and stirring for a further 24 hours. After 24 hours the reaction volume was diluted with 50 mL of ether. The aqueous and ether layers were separated. The aqueous layer was extracted with 2 × 50 mL of ethyl acetate. The organic layers were then combined and dried with anhydrous Na_2SO_4 . The solvent was removed *in vacuo* to afford (4-benzyloxycarbonylaminophenyl)acetic acid (**Appendix 3**) as a yellow/white powder. ^1H NMR data was in line with literature values. The desired product for use without further purification.

General Method III: Synthesis of imines

Method A for imine synthesis

Imines were synthesised *via* condensation of the appropriately substituted benzaldehyde (1 eq, 10 mmol) and 3,4,5-trimethoxyaniline (1 eq, 10 mmol, 1.83 g) in ethanol (40 mL) at 90 °C for 4 hours. A catalytic quantity of 0.1 M HCl was added dropwise at the beginning of the reaction. The reaction volume was then reduced to 10-20 mL *in vacuo*. The imine precipitated overnight and was purified by recrystallization in the minimum volume of hot ethanol. The final product was isolated by gravity filtration and dried under high vacuum.

Method B for imine synthesis

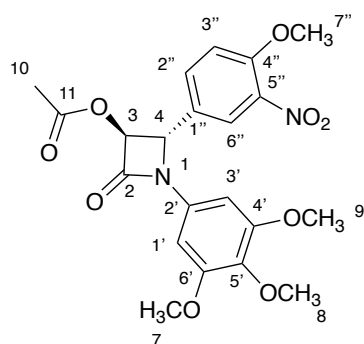
The appropriately substituted benzaldehyde (1 eq, 10 mmol) and 3,4,5-trimethoxyaniline (1 eq, 10 mmol, 1.83g) were added to minimum volume of cold ethanol at room temperature followed by addition of one drop of HCl (10% v/v). The reaction was stirred at room temperature until completion, indicated by TLC (1-2 hours). Imine products precipitated in cold ethanol when the reaction approached completion which were isolated *via* gravity filtration and washing with cold ethanol. Imines were dried overnight under high vacuum. Characterisation for the panel of imines (**44-56**) synthesised in this thesis is detailed in **Appendix 3**.

General Method IV: Synthesis of 3-acetoxy- β -lactams

Optimised Staudinger synthesis

To a stirring solution of acetoxyacetyl chloride (1.2 eq, 7 mmol, 0.78 mL) in an inert atmosphere in dry toluene (50 mL), the relevant imine (1 eq, 5 mmol) was added in stepwise portions. The reaction was heated to 100 °C and stirred for 20 minutes. A bright orange colour was observed on addition of imine to acetoxyacetyl chloride which slowly changed to bright yellow and subsequently pale yellow during the 20 minute reflux period. Anhydrous TEA (1.8 eq, 9mmol, 1.25 mL) was then added dropwise over 2-5 minutes under nitrogenous conditions. The reaction was stirred at 100 °C for a further 5 hours prior to stirring overnight at room temperature (16-24 hours). The reaction mixture was washed with dH₂O (2 × 30 mL) and the organic layer dried with anhydrous sodium sulphate (Na₂SO₄) before the solvent was removed under reduced pressure. The crude product was purified using flash column chromatography over silica gel using 1:1 *n*-hexane:ethyl acetate. Characterisation for the panel of 3-acetoxy -β-lactam racemates (**16a-22a**, **64a-66a**, **67a** and **67b**, **89-91**) is detailed in **Appendix 3**.

2-(4-Methoxy-3-nitrophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidine-3-yl acetate (63a)
63a was synthesised from **54** using **General Method IV** and isolated as a novel mixture of predominantly *trans* isomer in a ratio of 82:18 *trans:cis*



Yield: 1.33 g (2.98 mmol) 60 %

R_f: 0.2 (1:1 *n*-hexane:ethyl acetate)

Appearance: White solid crystalline powder

Melting Point: 171-172 °C

¹H NMR (400 MHz, CDCl₃): δ (mixture of *cis:trans* isomers 18:82) 7.89 (d, 1H, *J* = 2.4 Hz, H_{6''}), 7.83 (dd, 0.15H, (*cis*), *J* = 9.1 Hz, *J* = 1.9 Hz, H_{2''}), 7.54 (dd, 1H, *J* = 9.1 Hz, *J* = 1.9 Hz, H_{2''}), 7.51 (d, 0.15H, (*cis*), *J* = 2.4 Hz, H_{6''}), 7.14 (d, 1H, *J* = 8.6 Hz, H_{3''}), 7.10 (d, 0.15H, (*cis*), *J* = 8.6 Hz, H_{3''}), 6.54 (s, 0.3H, (*cis*), H_{1'&3'}), 6.50 (s, 2H, H_{1'&3'}), 5.93 (d, 0.11H, (*cis*), *J* = 4.8 Hz, H₃), 5.34 (d, 0.11H, (*cis*), *J* = 4.78 Hz, H₄), 5.30 (d, 1H, (*trans*), *J* = 2.4 Hz, H₃), 4.91 (d, 1H, (*trans*), *J* = 2.4 Hz, H₄), 3.98 (s, 3H, H_{7''}), 3.86 (s, 0.5H, (*cis*), H_{7''}), 3.78 (s, 3H, H₈), 3.75 (s, 1H, (*cis*), H_{7&9}), 3.73 (s, 6H, H_{7&9}), 2.22 (s, 0.4H, (*cis*), H₁₀), 2.21 (s, 3H, H₁₀)

¹³C NMR (100 MHz, CDCl₃): δ 160.9 (C₂), 153.8 (C_{4'&6'}), 153.4, 123.6, 132.6, 131.7, 127.7, 124.0, 114.5, 95.3 (C_{1'&3'}), 82.3 (C₃), 62.7 (C₄), 61.0, 56.7 (C_{7&9}), 56.3, 20.4 (C₁₀)

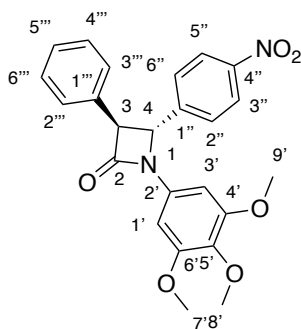
HRMS: APCI Calculated for C₂₁H₂₃N₂O₉ [M + H⁺] 447.139807, found 447.139619, error + 0.4 ppm

General Method V: Optimised synthesis for 3-substituted β -lactam racemates

To a stirring solution of the appropriate acid chloride (2 eq, 10 mmol), under a nitrogen atmosphere in anhydrous toluene (50 mL), relevant imine (5 mmol) was added. The reaction was heated to reflux at 100 °C and stirred at reflux for 10-15 minutes. Anhydrous TEA (2 eq, 10 mmol, 1.4 mL) was then added dropwise over 10 minutes under a nitrogen atmosphere. The reaction was stirred for 5 hours prior to cooling to room temperature and stirring overnight (16-24 hours). A characteristic darkening of the reaction was observed during reaction progression. The residue was diluted with dH₂O and the organic layer retained. The organic layer was then washed with a saturated solution of brine (2 $\times$ 30 mL) and dried with anhydrous Na₂SO₄, filtered and the solvent removed under reduced pressure to afford a crude yellow/brown sticky oil. The crude product was purified using gradient flash column chromatography over silica gel 5:1 to 1:1 *n*-hexane:ethyl acetate. The product was washed with a few drops of diethyl ether and placed on high vacuum to give an amorphous white/yellow powder of respective β -lactam. Characterisation for the panel of 3-substituted racemates **31**, **34** and **70-75** are reported in Appendix 3.

3-Phenyl-4-(*p*-tolyl)-1-(3,4,5-trimethoxyphenyl)azetidine-2-one (**91**)

91 was synthesised from **52** and phenylacetyl chloride using General Method Vb.



Yield: 710 mg (1.64 mmol) 48 %

Appearance White crystalline powder

R_f: 0.35 (1:1 *n*-hexane: ethyl acetate)

Melting Point: 146-148 °C

¹H NMR (400 MHz, CDCl₃): δ 8.32 (d, 2H, J = 8.4 Hz, H_{3''&5''}), 7.61 (d, 2H, J = 8 Hz, H_{2''&6''}), 7.42 (d, 2H, J = 7.6 Hz), 6.57 (s, 2H, H_{1&3}), 5.06 (d, 1H, J = 2.2 Hz, H₄), 4.23 (d, 1H, J = 2.2 Hz, H₄), 3.81 (3H, H_{8'}), 3.76 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz, CDCl₃): δ 164.6 (C₂), 153.8 (C_{4'&6'}), 148.2, 144.9, 135.1 (C_{2'}), 133.8 (C_{5'}), 133.1, 129.3, 128.4, 127.4, 126.8, 124.7, 94.9 (C_{1'&3'}), 65.2 (C₄), 63.1, 61.0 (C_{8'}), 56.2 (C_{7'&9'})

IR ν_{max} (ATR): 1745 cm⁻¹ (β -lactam C=O), 1506 cm⁻¹ (N-O)

HRMS: APCI calculated for C₂₄H₂₃N₂O₆ [M+H⁺] 435.155063; found 435.155063, s -2.1 ppm

General Method VI: Synthesis of 3-phenolic β -lactams using triphosgene-mediated acid activation of substituted acetic acids

A mixture of the relevant substituted acetic acid (1.5 eq, 15 mmol) and triphosgene [bis(trichloromethyl)carbonate] (0.5 eq, 5 mmol, 1.49 g, 0.834 mL) was added to 50 mL of anhydrous toluene and heated to 100 °C. The substituted acetic acid was activated to the respective acid chloride by stirring under reflux for 30 minutes with triphosgene. Next a solution of the appropriately substituted imine (1 eq, 10 mmol) dissolved in anhydrous toluene was injected dropwise to the refluxing solution. This was followed by the addition of anhydrous triethylamine (3 eq, 30 mmol, 5 mL). The reaction was refluxed for a further 5 hours before cooling to room temperature and stirring overnight. The mixture was washed with dH₂O (2 × 50 mL) and NaHCO_{3(sat)} (2 × 50 mL). The organic layer was retained and dried using anhydrous Na₂SO₄. The crude product was then purified using LC with isocratic (80:20 *n*-hexane: ethyl acetate) to afford protected 3-phenolic products. Characterisation for benzyloxy protected phenolic racemates **79-81** are presented in **Appendix 3**.

General Method VII: Synthesis of β-lactam derivatives via Reformatsky reaction

Method A: Reflux

Zinc dust (900 mg, 2 eq, 13.8 mmol) was suspended in a stirring solution of anhydrous benzene (20 mL) under a nitrogenous atmosphere. The zinc catalyst was activated using trimethylchlorosilane (0.91 mL, 1 eq, 7.1 mmol) by stirring at 40 °C for 15 minutes, followed by heating to reflux (~ 85-88 °C) and stirring vigorously for 5 minutes. The reaction vessel was then cooled on ice. Imine **47** (1 eq, 7mmol, 3.0g) was added on ice at 0 °C followed by the respective substituted ethyl bromoacetate (1.8 eq, 12.6 mmol). A vigorous effervescence was observed. The reaction was then heated to reflux temperatures for 8 hours before cooling to room temperature and allowing to stir overnight. The zinc catalyst was removed from solvent by filtration through Celite. The reaction was diluted with dichloromethane (3 × 25 mL) followed by filtration through Celite. The filtrate was retained and washed with NH₄Cl_(sat) (40 mL), 0.1M HCl (40 mL) and brine (40 mL). The organic layer was dried with anhydrous Na₂SO₄, filtered and solvent subsequently reduced *in vacuo* to yield a brown/black gum. The pure product was isolated using flash chromatography over silica gel with isocratic conditions of 4:1 *n*-hexane: ethyl acetate.

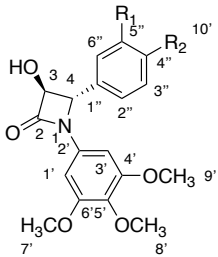
Method B: Microwave technology

Zinc powder (4.5 eq, 9 mmol, 0.588 g) was activated using trimethylchlorosilane (3.5 eq, 7 mmol, 0.76g, 0.65mL) in anhydrous benzene (4 mL) by heating for 15 min at 40 °C and subsequently for 2 min at 100 °C with microwave irradiation. After cooling the reaction vessel on ice, the imine **03ii** (1 eq, 2 mmol, 0.86 g) was added. Next the appropriate substituted ethyl bromoacetate (2.5 eq, 5mmol) was added to the reaction vessel and the mixture was placed in

the microwave reactor for 30 min at 100 °C. The reaction mixture was filtered through Celite to remove the zinc catalyst and the residue washed with DCM (30 mL). The filtrate was further diluted with DCM (30 mL). This solution was washed with saturated ammonium chloride solution (20 mL), 25 % ammonium hydroxide (20 mL), 0.1 HCl (40 mL), followed by dH₂O (40 mL). The organic phase was dried over anhydrous Na₂SO₄ and the solvent was removed by evaporation *in vacuo*. A black gum was obtained as the crude product. The purified product was isolated from the crude gum by flash column chromatography over silica gel (eluent: hexane: ethyl acetate gradient). Characterisation for silyl ether protected Reformatsky products are detailed in **Appendix 3**.

General Method VIII: Synthesis of 3-hydroxy- β -lactam racemates *via* hydrazinolysis to the corresponding acetate ester

Hydrazine dihydrochloride (2 eq) was added to a stirred solution of *trans* 3-acetoxy- β -lactam intermediates (1 eq) in anhydrous methanol (30mL) under a nitrogenous atmosphere and on ice at 0 °C. Anhydrous TEA (2.2 eq) was then added dropwise. The mixture was then allowed to reach room temperature before heating to reflux and stirring at 85 °C for 4-6 hours. The solvent was then removed *in vacuo* and the residue diluted with KHSO_{4(sat)}. The product was extracted with ethyl acetate (2 X 50 mL). The combined organic layers were dried with anhydrous Na₂SO₄ and subsequently filtered and solvent removed under reduced pressure. The crude residue was purified by flash chromatography over silica gel (eluent *n*-hexane: ethyl acetate; 1:2) to afford the pure product. Previously reported racemates are detailed in **Appendix 3** while characterisation for novel racemates is detailed below.

		
Racemate code	R ₁	R ₂
63	NO ₂	OCH ₃
89	H	F
90	H	NO ₂

The remainder of 3-hydroxyl racemates are listed in Appendix 3.

4-(4-Fluorophenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (89)

89 was obtained from **89a** by **General Method VIII**.

Overall yield for Staudinger: 350 mg (1.01 mmol) 11%

R_f: 0.31 (1:1; *n*-hexane:ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 7.35 (apparent m, 2H, $J_{5''3''-2''6''} = 8$ Hz, $J_{5''3''-F} = 14.6$ Hz, H_{5''&3''}), 7.12 (apparent triplet, 2H, $J_{2''6''-F} = 17.5$ Hz, $J_{2''6''-5''3''} = 8$ Hz), 6.49 (s, 2H, H_{1'&3'}), 4.86 (d, 1H, $J = 2.1$ Hz, H₃), 4.75 (d, 1H, $J = 2.1$ Hz, H₄), 3.78 (s, 3H, H₈), 3.71 (s, 6H, H_{7&9})

¹³C NMR (100 MHz, CDCl₃): δ 166.1 (C₂), 164.2, 161.7, 153.5 (C_{4'&6'}), 134.8 (C_{2'}), 133.2 (C_{5'}), 128.0, 116.4 (C_{5''&3''}), 116.2 (C_{2''&6''}), 95.5 (C_{1'&3'}), 83.9 (C₃), 65.1 (C₄), 61.0 (C_{7&8}), 56.1 (C_{7'&9'})

¹⁹F NMR (376 MHz, CDCl₃): δ -112.1

HRMS: APCI calculated for C₁₈H₁₉FNO₅ [M+H⁺] 348.124177; found 348.12425, error +0.2 ppm

3-Hydroxy-4-(4-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (90) *trans*

90 was synthesised using **90a** using **General Method VIII** and isomers purified using LC over silica gel with isocratic conditions of 1:1 *n*-hexane: ethyl acetate.

Yield: 113 mg (0.3 mmol) 6% as *trans* isomer, 100 mg (0.28 mmol) 11% as mixed isomers

Melting Point: 162-167 °C

R_f: 0.13 (*trans*) (1:1; *n*-hexane:ethyl acetate), 0.34 (1:2; *n*-hexane:ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 8.29 (d, 2H, $J_{5''3''-2''6''} = 9.1$ Hz, H_{5''&3''}), 7.55 (d, $J_{2''6''-5''3''} = 9.1$ Hz, 2H, H_{2''&6''}), 6.45 (s, 2H, H_{1'&3'}), 4.98 (d, 1H, $J_{3-4} = 1.8$ Hz, H₃), 4.75 (d, 1H, $J_{4-3} = 1.9$ Hz, H₄), 3.77 (s, 3H, H₈), 3.72 (s, 6H, H_{7&9})

¹³C NMR (100 MHz, CDCl₃): δ 165.3 (C₂), 153.7 (C_{4'&6'}), 148.3 (C_{1'}), 135.3 (C_{2'}), 132.6 (C_{5'}), 126.9 (C_{3''&5''}), 124.6 (C_{2''&6''}), 95.3 (C_{1'&3'}), 83.8 (C₄), 64.8 (C₃), 61.0 (C₈), 56.2 (C_{7'&9'})

IR ν_{max} (ATR): 3287 cm⁻¹ (OH), 1724.1 cm⁻¹ (β-lactam C=O), 1508 cm⁻¹ (N-O)

HRMS: APCI calculated for C₁₈H₁₇N₂O₇ [M-H⁺] 373.104124; found 373.103566, error -1.5 ppm

3-Hydroxy-4-(4-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (90) *cis*

90 cis was synthesised from **90a** using **General Method VIII** and using LC with isocratic conditions (1:1 *n*-hexane:ethyl acetate).

Yield: 30 mg (0.08 mmol) 1.6% as *cis* isomer

R_f: 0.07 (*cis*) (1:1; *n*-hexane:ethyl acetate), 0.22 (1:2; *n*-hexane:ethyl acetate), 0.33 (1:3; *n*-hexane:ethyl acetate)

Melting Point: 165 °C

¹H NMR (400 MHz, CDCl₃): δ 8.30 (d, 2H, $J_{5''3''-2''6''} = 9.1$ Hz, H_{5''&3''}), 7.56 (d, $J_{2''6''-5''3''} = 9.1$ Hz, 2H, H_{2''&6''}), 6.57 (s, 2H, H_{1'&3'}), 5.38 (d, 1H, $J_{3-4} = 3.8$ Hz, H₃), 5.34 (d, 1H, $J_{4-3} = 3.8$ Hz, H₄), 3.81 (s, 3H, H₈), 3.76 (s, 6H, H_{7&9})

¹³C NMR (100 MHz, CDCl₃): δ 165.2 (C₂), 153.8 (C_{4'&6'}), 148.3 (C_{1'}), 140.9, 135.5 (C_{2'}), 132.6 (C_{5'}), 128.6 (C_{3''&5''}), 124.0 (C_{2''&6''}), 95.2 (C_{1'&3'}), 77.5 (C₃), 61.74 (C₄), 61.8 (C₈), 56.0 (C_{7'&9'})

IR ν_{max} (ATR): 3435 cm⁻¹ (OH), 1747.1 cm⁻¹ (β-lactam C=O), 1508 cm⁻¹ (N-O)

HRMS: APCI calculated for C₁₈H₁₇N₂O₇ [M-H⁺] 373.104124; found 373.104122, error 0.0 ppm

3-Hydroxy-4-(4-methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (63)

63 was synthesised from **63a** using **General Method VIII**

Yield: 330 mg (0.82 mmol), 27.3 % for hydrazinolysis

Overall yield from Staudinger: 16.4%

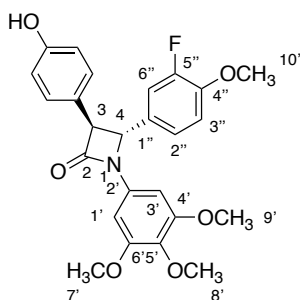
¹H NMR (600 MHz, CDCl₃): δ 7.86 (d, 1H, *J* = 1.9 Hz, H_{6''}), 7.68 (bs, 1H, OH), 7.48 (dd, 1H, *J* = 9.3 Hz, *J* = 2.6 Hz, H_{2''}), 7.11 (d, 1H, *J* = 8.9 Hz, H_{3''}), 6.43 (s, 2H, H_{1'&3'}), 4.83 (d, 1H, *J* = 1.9 Hz, H₃), 4.72 (d, 1H, *J* = 1.9 Hz, H₄), 3.97 (s, 3H, H_{10'}), 3.77 (s, 3H, H_{8'}), 3.72 (s, 6H, H_{7'&9'})
¹³C NMR (100 MHz, CDCl₃): δ 169.9, 161.0, 153.8, 153.4, 139.9, 135.4, 132.3, 131.8, 131.7, 127.6, 123.9, 114.4, 95.5, 82.4, 62.5, 60.9, 56.7, 56.3
¹⁵N NMR (60.8 MHz; CDCl₃): 367.2 (NO₂), 155.7 (β-lactam ring)
HRMS: APCI Calculated for C₁₉H₂₁N₂O₈ [M +H⁺] 405.12942, found 405.129640 error -1.0 ppm

General Method IX: Debenzylation of 80-81 to afford free 3-phenolic β-lactams 68-89

The respective benzyl ether protected β-lactam was dissolved in a 1:1 mixture of ethanol/ethyl acetate and was hydrogenated over palladium on carbon (10%) at atmospheric pressure until completion as monitored by TLC (2.5 hours). The catalyst was then filtered away using Celite and washed with ethyl acetate before the residual solvent was removed under vacuum. No further purification of the resulting product was required. Characterisation for previously reported intermediates are detailed in **Appendix 3** while novel racemates are detailed below.

4-(3-Fluoro-4-methoxyphenyl)-3-(4-hydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (68)

68 was synthesised from **80** using **General Method IX**.



Yield: 550mg (1.21 mmol) 78% for debenzylation, 18.4% overall for Staudinger

Appearance: white/grey fluffy solid.

R_f: 0.42 (1:1 *n*-hexane: ethyl acetate)

Melting Point: 95°C

¹H NMR (600 MHz, DMSO-*d*₆): δ 9.55 (bs, 1H, OH) 7.40 (dd, 1H, *J* = 1.8 Hz, *J* = 9 Hz, H_{2''}), 7.30 (d, 1H, *J* = 8.7 Hz, H_{3''}), 7.19 (apparent triplet, 1H, *J* = 9.1 Hz), 7.13 (d, 2H, *J* = 9 Hz), 6.78 (d, 2H, *J* = 9 Hz), 6.59 (s, 2H, H_{1&3}), 5.14 (d, 1H, *J* = 1.9 Hz, H₃), 4.34 (d, 1H, *J* = 1.9 Hz, H₄), 3.83 (s, 3H, OCH₃, H_{10'}), 3.65 (s, 6H, H_{7'&9'}), 3.58 (s, 3H, OCH₃, H_{8'})

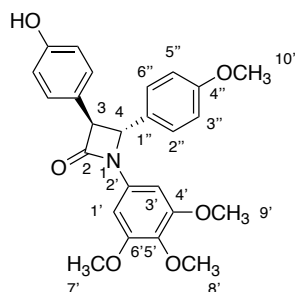
¹³C NMR (100 MHz, DMSO-*d*₆): δ 166.4, 157.8, 154.4, 153.3, 150.8, 147.5, 147.6, 134.4, 133.5, 130.8, 129.2, 125.3, 123.9, 128.1, 127.5, 123.9, 116.1, 114.8, 114.6, 95.5, 63.53 (C_{3''}), 62.3 (C_{1''}), 60.6 (C₈), 60.3, 56.5, 56.2

¹⁹F NMR (376 MHz, DMSO-*d*₆): δ -135.2

IR ν_{max}(ATR): 3298 cm⁻¹ (OH), 1726 cm⁻¹ (β-lactam C=O)

HRMS: (APCI) Calculated for $C_{25}H_{23}FNO_6$ [$M - H^+$] 452.151489, found 452.152126, error +1.4 ppm, Calculated for $C_{25}H_{25}FNO_6$ [$M + H^+$] 454.1666042, found 454.165455, error -1.3 ppm, Calculated for $C_{25}H_{24}FNNaO_6$ [$M + Na^+$] 476.147986, found 476.148814, error -1.7 ppm

(3-(4-Hydroxyphenyl)-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (69)
69 was synthesised from **81** using **General Method IX**.



Yield: 330mg (0.65 mmol), 13% yield from Staudinger

R_f: 0.29 (1:1 *n*-hexane: ethyl acetate)

Appearance: grey powder

¹H NMR (600 MHz, CDCl₃): δ 7.35 (d, 2H, *J* = 7.8 Hz, H_{2''}&6''), 7.19 (d, 2H, *J* = 7.8 Hz), 6.96 (d, 2H, *J* = 7.8 Hz), 6.83 (d, 2H, *J* = 7.8 Hz), 6.62 (s, 2H, H_{1'}&3'), 4.82 (d, 1H, *J* = 2.2 Hz, H₄), 4.22 (d, 1H, *J* = 2.2 Hz, H₄), 3.85 (s, 3H, H_{10'}), 3.79 (s, 3H, H_{8'}), 3.75 (s, 7H, H_{7'}&9')

¹³C NMR (100 MHz, CDCl₃): δ 166.3 (C₂), 159.9 (C_{4''}), 155.6, 153.5 (C_{4'}&6'), 134.5 (C_{2'}), 133.7 (C_{5'}), 130.5, 129.3, 128.7, 127.3, 126.6, 115.9, 115.9, 113.8, 94.9 (C_{1'}&3'), 64.5 (C₄), 64.3 (C₃), 61.0 (C_{8'}), 56.1 (C_{7'}&9'), 56.0 (C_{10'})

HRMS: (APCI) Calculated for $C_{25}H_{23}FNO_6$ [$M - H^+$] 452.151489, found 452.152126, error +1.4 ppm, Calculated for $C_{25}H_{25}FNO_6$ [$M + H^+$] 454.1666042, found 454.165455, error -1.3 ppm, Calculated for $C_{25}H_{24}FNNaO_6$ [$M + Na^+$] 476.147986, found 476.148814, error -1.7 ppm

General Method X: Desilylation of β-lactams

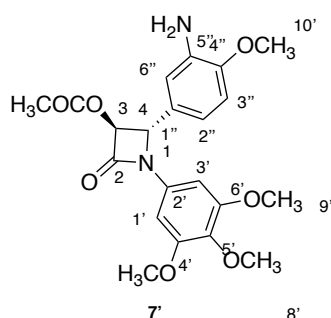
To a stirring solution of TBDMS protected phenol (1 eq) in anhydrous THF (30 mL), TBAF, 1M in THF (1.5 eq) was added dropwise at 0 °C under a nitrogenous atmosphere. The reaction mixture was allowed to stir until the reaction had reached completion as monitored and indicated *via* TLC (2-3 hours). The solution was diluted with ethyl acetate (50 mL) which was then washed with 0.1M HCl (100 mL). The aqueous layer was then extracted using ethyl acetate (2 X 25 mL). The combined organic layers were then washed with brine (100 mL), water (100 mL) and dried over anhydrous Na₂SO₄, filtered and solvent removed under reduced pressure. The crude product was subjected to column chromatography under gradient elution (1:1 to 1:2 hexane:ethyl acetate) to afford the pure phenolic product. Characterisation for previously reported B ring *meta* hydroxy substituted racemates are reported in **Appendix 3**. Novel characterisation is reported below.

General Method XI: Reduction of -NO₂ to -NH₂ group

The appropriate β -lactam derivative (1 eq) was suspended in glacial acetic acid (5-10 mL) under a nitrogenous atmosphere. Metallic zinc dust (10 eq) was added to the stirring suspension and stirred for 6 days. On completion, the reaction was diluted with DCM (20-50 mL) and filtered through celite to remove the zinc dust. The dichloromethane layer was washed with dH₂O (2 $\times$ 20 mL) to remove the glacial acetic acid. The amine derivative was then isolated using flash chromatography over silica gel using a gradient of *n*-hexane: ethyl acetate (3:1-1:2). Previously reported B ring *meta* NH₂ substituted racemates are reported in **Appendix 3**.

4-(3-Amino-4-methoxyphenyl)-3-(methylperoxy)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (64a)

64a was synthesised from **63a** using General Method XI.



Yield: <15%

R_f: 0.25 (1:4 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 8.55 (d, 1H, *J* = 1.8 Hz, H_{6''}), 7.81 (bs, 1H, NH₂), 7.02 (dd, 1H, *J* = 1.8 Hz, *J* = 8.6 Hz, H_{2''}), 6.89 (d, 1H, *J* = 8.6 Hz, H_{3''}), 6.59 (s, 2H), 5.49 (d, 1H, *J* = 1.1 Hz, H₃), 4.90 (d, 1H, *J* = 1.1 Hz, H₄), 3.92 (s, 3H), 3.78 (s, 3H), 3.72 (s, 6H), 2.20 (s, 3H, OCOCH₃).

¹³C NMR (100 MHz, CDCl₃): δ not determined

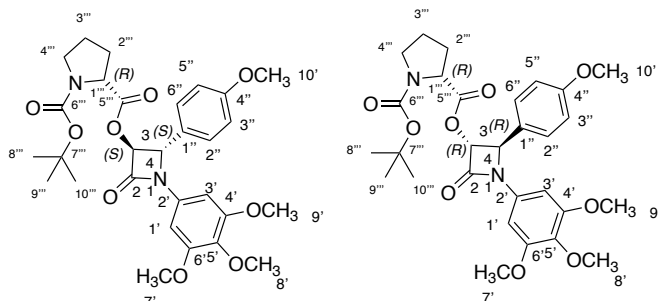
General Method XII: Diastereomer derivatisation using *N*-(Boc)-L-proline

To a stirring solution of *trans* β -lactam racemate (1 eq) in anhydrous MeCN (30 mL) under a nitrogenous atmosphere, HBTU (2.2 eq) was added. *N*-(*tert*-Butoxycarbonyl)-L-Proline (2 eq) was then added followed by addition of DIPEA (30 eq). The solution was stirred for 24 hours at room temperature prior to removal of the solvent *in vacuo*. The residue was diluted with dH₂O and extracted with DCM (3 $\times$ 30 mL). The combined organic layers were washed with potassium bisulfate (50 mL), sodium hydrogen carbonate (50 mL) and brine (50 mL). The organic layer was dried with anhydrous Na₂SO₄ before filtration and removal of organic solvent under reduced pressure. The crude residue was then purified by flash chromatography over silica gel using gradient elution; 4:1 to 3:2 *n*-hexane:ethyl acetate affording a pure mixture of diastereomers in high yield (75-80%). The purified diastereomers were analysed using TLC with various ratios of MTBE: *n*-hexane. In most cases separation of diastereomers was not observed and thus no further purification was carried out. Diastereomer derivatives are therefore characterised and reported as the diastereomer mixture. A range of proline

diastereomers were successfully resolved using LC with gradient elution of 4:1 – 1:4 *n*-hexane: MTBE and are characterized below.

1-(*Tert*-butyl) 2-(2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*R*)-pyrrolidine-1,2-dicarboxylate (107)

107 was synthesised from **17** and *N*-Boc-D-proline using **General Method XII**.



Yield: 200 mg (0.36 mmol) 52 %

Appearance: white amorphous powder/ gel

R_f: 0.53 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (600 MHz, CDCl₃): δ (diastereomers and rotamers present) 7.33-7.29 (m, 2H, H_{2''}&6''), 6.96-6.9 (m, 2H, H_{3''}&5''), (6.56, 6.55, 6.55, 6.55 (s, 2H), H_{1'}&3'), 5.45 (apparent s, 0.2H, H₃), 5.41 (apparent s, 0.2H, H₃), 5.4 (d, 0.16H, *J* = 1.8 Hz, H₃), 5.4 (d, 0.3H, *J* = 1.8 Hz, H₃), 5.34 (d, 0.2H, *J* = 1.8 Hz, H₃), 5.0 (apparent s, 0.3H, H₄), 4.94 (apparent s, 0.3H, H₄), 4.89 (d, 0.2H, *J* = 1.8 Hz, H₄), 4.86 (apparent s, 0.35H, H₄), 4.42 – 4.37 (m, 1H, H_{1''}), (3.85, 3.85, 3.84, 3.83, 3.82, 3.82 (s, 3H), H_{10'}), (3.79, 3.78, (s, 3H), H_{8'}), (3.72, 3.72 (s, 6H), H_{7'}&9'), 3.66-3.40 (m, 2H, proline ring), 2.36-2.25 (m, 1.5H, proline ring), 2.1 – 1.9 (m, 3H, proline ring), 1.52 (s, 3.5H, *t*-butyl), 1.47 (s, 2H, *t*-butyl), 1.45 (s, 3.5H, *t*-butyl).

¹³C NMR (100 MHz, CDCl₃): δ 172.4, 172.3, 172.1, 172.0, 169.7, 161.7, 161.5, 161.4, 161.2, 160.3, 160.2, 160.1, 160.0, 157.7, 154.5, 153.6, 153.5, 135.1, 135.0, 134.9, 134.9, 133.1, 133.1, 133.0, 132.9, 128.0, 127.9, 127.8, 127.7, 127.7, 127.1, 127.0, 126.8, 114.7, 114.6, 114.6, 114.5, 95.5, 95.4, 95.3, 82.9, 82.8, 82.7, 82.5, 80.4, 80.2, 80.1, 65.9, 63.7, 63.6, 63.5, 63.4, 61.2, 61.0, 59.4, 59.1, 58.8, 58.7, 58.6, 56.3, 56.1, 55.4, 47.3, 46.7, 46.7, 46.4, 34.5, 31.1, 31.0, 29.0, 29.9, 28.5, 28.4, 28.4

¹H NMR (600 MHz, DMSO-*d*₆): δ 7.44 (m, 1H), 7.41 (m, 1H), 6.97 (m, 2H), 6.56 (s, 0.35H), 6.54 (s, 0.5H), 6.53 (s, 1H), 5.57 (d, 0.2H, *J* = 1.8 Hz), 5.56 (d, 0.2H, *J* = 1.8 Hz), 5.51 (d, 0.2H, *J* = 1.8 Hz), 5.50 (d, 0.2H, *J* = 1.8 Hz), 5.45 (d, 0.2H, *J* = 1.8 Hz), 5.52 (apparent s, 0.4H), 5.17 (d, 0.4H, *J* = 1.8 Hz), 5.13 (apparent s, 0.3H), 4.32-2.29 (m, 1H), 3.75 (s, 3H), 3.63 (s, 6H), 3.58 (s, 3H), 2.32-2.23 (s, 2H), 2.03-1.78 (m, 4H), (1.42, 1.38, 1.35, 1.34 (s, 9H).

¹³C NMR (100 DMSO-*d*₆): δ 174.8, 174.4, 172.4, 172.3, 172.2, 172.0, 169.9, 162.0, 161.6, 161.5, 160.2, 160.1, 160.1, 154.1, 154.0, 154.0, 153.7, 153.2, 134.8, 132.8, 132.8, 132.7, 129.1, 129.0, 129.0, 129.0, 128.9, 127.9, 127.7, 127.6, 127.6, 12.6, 114.8, 96.1, 96.0, 96.0, 92.1, 92.1, 82.3, 82.2, 82.2, 82.1, 79.7, 79.7, 79.1, 65.4, 62.7, 62.6, 62.5, 62.5, 62.4, 61.0, 59.1, 58.8, 58.8, 58.8, 58.7, 58.7, 55.6, 46.9, 46.7, 30.9, 30.8, 29.8, 28.59, 28.5, 28.5, 28.4, 28.4, 24.5, 23.7

HRMS: APCI Calculated for C₂₉H₃₆N₄O₉ [M+ Na⁺] 579.231301, found 579.230838, error + 1.5 ppm

Purity RP-HPLC: 89 -90%

1-(*Tert*-Butyl) 2-((2*S*,3*S*)-2-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*R*)-pyrrolidine-1,2-dicarboxylate (107a)

107a was resolved from **107a** using LC with a gradient of 4:1 to 1:4 *n*-hexane: MTBE.

Yield: 30 mg (0.083 mmol) 23.2%

Appearance: white fluffy powder

R_f: 0.33 (4:6 *n*-hexane: MTBE), plate developed twice

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 7.31 (m, 2H), 6.93 (m, 2H), (6.56, 6.56, 6.55, (s, 2H)), 5.41 (d, 0.08H, *J* = 1.8 Hz), 5.4 (apparent s, 0.25H), 5.4 (d, 0.2H, *J* = 1.8 Hz), 5.4 (d, 0.3H, *J* = 1.8 Hz), 5.34 (d, 0.1H, *J* = 1.8 Hz), 5.0 (apparent s, 0.35H), 4.94 (apparent s, 0.1H), 4.89 (d, 0.2H, *J* = 1.8 Hz), 4.86 (apparent s, 0.3H), 4.39 (m, 1H), 3.83 (s, 1.7H), 3.81 (s, 1.3H), 3.79 (s, 2.2H), 3.78 (s, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.62-3.4 (m, 2H), 2.35-2.2 (m, 2H), 2.1-1.9 (m, 2H), 1.52 (s, 5H), 1.48 (s, 3H), 1.43 (s, 1H)

¹³C NMR (100 MHz, CDCl₃): δ 172.1, 171.9, 161.5, 161.2, 160.3, 160.0, 154.5, 153.6, 153.5, 153.6, 153.5, 153.4, 135.1, 134.9, 133.1, 133.0, 127.1, 126.8, 114.7, 114.6, 95.5, 95.4, 82.8, 82.7, 80.4, 80.1, 65.9, 63.7, 63.5, 63.4, 63.4, 61.0, 59.4, 59.2, 58.8, 58.5, 56.9, 56.1, 55.4, 46.7, 46.4, 31.1, 31.0, 29.9, 29.7, 28.5, 28.4, 24.6, 23.7

¹H NMR (600 MHz, DMSO-*d*₆): δ 7.44 (d, 1.4H, *J* = 8.9 Hz), 7.42 (d, 0.6H, *J* = 8.9 Hz), 6.98 (m, 2H), 6.55 (s, 0.7H), 6.52 (s, 1.3H), 5.57 (s, 0.5H, *J* = 1.8 Hz), 5.51 (s, 0.08H, *J* = 1.8 Hz), 5.50 (d, 0.2H, *J* = 1.8 Hz), 5.5 (d, 0.1H, *J* = 1.8 Hz), 5.22 (d, 0.2H, *J* = 1.8 Hz), 5.17 (d, 0.7H, *J* = 1.8 Hz), 4.29 (m, 1H), 3.79 (s, 3H), 3.36 (s, 6H), 3.6 (s, 3H), 2.36-2.25 (m, 2H), 1.98-1.78 (m, 4H), 1.42 (s, 2.5H), 1.42 (s, 2H), 1.40 (s, 1H), 1.38 (s, 1H), 1.34 (s, 4H), 1.34 (s, 2H)

¹³C NMR (100, DMSO-*d*₆): δ 172.3, 161.5, 160.1, 153.4, 153.0, 147.6, 146.2, 136.4, 135.0, 132.8, 129.0, 114.71, 102.5, 95.96, 82.3, 79.71, 62.6, 60.48, 59.3, 58.8, 56.31, 55.7, 46.8, 30.6, 28.2, 28.33, 23.68 **HRMS: APCI** Calculated for C₂₉H₃₅N₂O₉ [M + H⁺] 555.23404, found 555.232029, error -5.0 ppm

IR ν_{max}(ATR): 1756 cm⁻¹ (β-lactam C=O), 1694 cm⁻¹ (*N*-Boc-D-proline C=O)

Purity RP-HPLC: 85%

1-(*Tert*-butyl) 2-((2*R*,3*R*)-2-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*R*)-pyrrolidine-1,2-dicarboxylate (107b)

107b was resolved from **107** using LC with a gradient of 4:1 to 1:4 *n*-hexane: MTBE.

Yield: not calculated

Appearance: white fluffy powder

R_f: 0.30 (4:6 *n*-hexane: MTBE), plate developed twice

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 7.30 (m, 2H), 6.89 (apparent triplet, 2H, *J* = 9.6 Hz), 6.56 (s, 2H), 5.4 (apparent s, 0.5H), 5.35 (apparent s, 0.5H), 5.1 (apparent s, 0.1H), 4.93 (apparent s, 0.3H), 4.86 (apparent s, 0.4H), 4.39 (m, 1H), 3.84 (s, 2H), 3.82 (s, 1H), 3.78 (s, 3H), 3.72 (s, 6H), 3.62-3.4 (m, 2H), 2.39-1.89 (m, 4H), 1.53 (s, 3H), (1.48, 1.47 (s, 3H)), 1.43 (s, 5H)

¹H NMR (600 MHz, DMSO-*d*₆): δ 7.45 (d, 1H, *J* = 8.6 Hz), 7.41 (1H, *J* = 8.9 Hz), 6.97 (d, 2H, *J* = 8.1 Hz), 6.54 (s, 0.3H), 6.54 (s, 0.9H), 6.53 (s, 0.8H), 6.55 (d, 0.5H, *J* = 1.8 Hz), 5.51 (d, 0.6H, *J* = 1.8 Hz), 5.21 (d, 0.5H, *J* = 1.8 Hz), 5.17 (d, 0.3H, *J* = 1.8 Hz), 5.14 (d, *J* = 0.3H), 4.32 (m, 1H), 3.73 (s, 3H), 3.63 (s, 6H), 3.58 (s, 3H), 2.33-2.16 (m, 3H), 2.04-1.7 (m, 4H), 1.39 (s, 2H), 1.38 (s, 2H), 1.34 (s, 3H), 1.32 (s, 3H).

¹³C NMR (100 DMSO-*d*₆): δ 174.9, 174.4, 172.3, 172.0, 171.0, 161.7, 154.0, 153.7, 153.6, 153.2, 134.9, 132.8, 132.7, 129.6, 129.1, 128.9, 127.6, 127.5, 114.7, 114.6, 114.7, 96.1, 96.0,

94.0, 92.2, 92.1, 82.2, 82.2, 79.7, 79.7, 78.9, 78.5, 65.4, 63.6, 62.8, 62.4, 60.6, 60.5, 59.1, 58.7, 57.3, 57.1, 56.2, 56.0, 46.8, 46.7, 46.7, 46.6, 37.2, 35.3, 28.5, 28.4, 28.4, 24.9

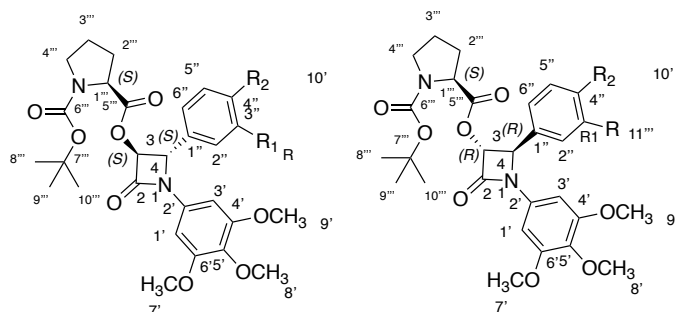
IR $\nu_{\max}$ (ATR): 1756 (β -lactam C=O) cm^{-1} , 1694 cm^{-1} (*N*-Boc-D-proline C=O)

HRMS: APCI calculated for $\text{C}_{29}\text{H}_{35}\text{N}_2\text{O}_9$ [$\text{M} - \text{H}^+$], 555.234804; found 555.231776, error +5.5 ppm

ESI calculated for $\text{C}_{29}\text{H}_{36}\text{N}_2\text{NaO}_9$ [$\text{M} + \text{Na}^+$], 579.231301; found 579.231808, error -0.9 ppm

Purity RP-HPLC: 95%

1-(*Tert*-butyl) 2-(2-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (108)



A mixture of diastereomers **108** was synthesised from **17** and *N*-Boc-L-proline using **General Method XII**.

Yield: 1.08g (1.7 mmol) 70-77%

Appearance: white amorphous powder

R_f: 0.53 (1:1 *n*-hexane: ethyl acetate)

Melting Point: 65-69 °C

^1H NMR (400 MHz, CDCl_3): δ 7.32 (doublet, 2H, $J = 8.9$ Hz, $\text{H}_{2''\&6''}$), 6.95 (d, 1.2H, $J = 8.9$ Hz, $\text{H}_{3''\&5''}$), 6.91 (d, 0.8H, $J = 8.9$ Hz, $\text{H}_{3''\&5''}$), 6.57 (s, 0.7H, $\text{H}_{1'\&3'}$), 6.56 (s, 1.3H, $\text{H}_{1'\&3'}$), 5.45 (d, 0.3H, $J = 1.9$ Hz, H_3), 5.41 (apparent s, 0.16H, H_3), 5.38 (d, 0.3H, $J = 1.9$ Hz, H_3), 5.34 (d, 0.2H, $J = 1.9$ Hz, H_3), 5.00 (apparent s, 0.25H, H_4), 4.94 (apparent s, 0.35H, H_4), 4.86 (apparent s, 0.5H, H_4), 4.40 (m, 1H, $\text{H}_{1''}$), 3.87 (s, 1.4H, $\text{H}_{10'}$), 3.83 (s, 0.1H, $\text{H}_{10'}$), 3.82 (s, 0.6H, $\text{H}_{10'}$), 3.79 (s, 1.2H, H_8), 3.79 (s, 1.7H, H_8), 3.72 (s, 6H, $\text{H}_{7'\&9'}$), 3.6-3.4 (m, 2H), 2.36-1.9 (m, 4H), 1.53 (s, 3H, *t*-butyl), 1.47 (s, 2.5H, *t*-butyl), 1.45 (s, 4.6H, *t*-butyl)

^{13}C NMR (100 MHz, CDCl_3): 172.4, 172.2, 172.0, 171.9, 161.5, 161.2, 160.3, 160.0, 154.5, 153.7, 153.6, 153.5, 135.0, 135.0, 134.9, 133.1, 133.0, 127.8, 127.0, 126.8, 116.2, 114.8, 114.6, 114.4, 112.0, 95.5, 95.3, 82.9, 82.8, 80.4, 80.3, 80.2, 63.7, 63.5, 63.4, 61.0, 59.1, 58.8, 58.6, 56.1, 55.4, 46.7, 46.4, 31.1, 30.1, 29.8, 24.6, 24.5, 23.7¹

^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.45 (d, 1.2H, $J = 7.8$ Hz), 7.40 (d, 0.8H, $J = 7.8$ Hz), 6.97 (d, 2H, $J = 7.8$ Hz), 6.54 (s, 0.1H), 6.53 (s, 1H), 5.58 (d, 0.1H, $J = 1.7$ Hz), 5.56 (d, 0.4H, $J = 1.7$ Hz), 5.52 (d, 0.3 H, $J = 1.7$ Hz), 5.50 (d, 0.1H, $J = 1.7\text{Hz}$), 5.21 (d, 0.3H, $J = 1.7\text{Hz}$), 5.17 (d, 0.2H, $J = 1.7\text{Hz}$), 5.13 (d, 0.4H, $J = 1.7\text{Hz}$), 4.31 (m, 1H), 3.75 (s, 3H), 3.63 (s, 6H), 3.58 (s, 3H), 3.45 (m, 2H), 2.28 (m, 1H), 2.00 (m, 1H), 1.87 (m, 2H), 1.42 (s, 1H), 1.38 (s, 3H), 1.35 (s, 2H), 1.34 (s, 4H).

^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 172.4, 172.3, 172.1, 172.0, 161.7, 161.5, 160.1, 154.1, 153.6, 153.2, 134.8, 132.8, 132.7, 129.1, 129.0, 128.9, 128.9, 127.7, 127.6, 127.5, 114.8, 114.7, 96.1, 96.0, 82.3, 82.1, 79.7, 79.6, 62.7, 62.6, 62.5, 60.6, 58.9, 58.7, 56.3, 55.6, 46.9, 46.7, 30.9, 30.8, 29.9, 29.8, 28.5, 28.3, 24.5, 23.7

IR $\nu_{\max}$ (ATR): 1756 cm^{-1} (β -lactam C=O), 1694 cm^{-1} (*N*-Boc-L-proline C=O)

HRMS: APCI calculated for $C_{29}H_{37}N_2O_9$ [$M + H^+$], 557.248291; found 557.24937, error -1.9 ppm. **ESI** calculated for $C_{29}H_{36}N_2NaO_9$ [$M + Na^+$], 579.23239; found 579.2313, error + 1.9 ppm
Purity RP-HPLC: >99%

1-(*Tert*-Butyl) 2-((2*S*,3*S*)-2-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (108a)

108a was resolved from **108** using LC with a gradient of *n*-hexane: MTBE

Yield: 300mg (0.54 mmol) 30%

Appearance: white amorphous powder

Melting Point: 66-72°C

R_f: 0.38 (1:2 *n*-hexane: MTBE), *plate developed twice*

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 7.23 (d, 2H, *J* = 8 Hz), 6.94 (d, 1H, *J* = 8.3 Hz), 6.90 (d, 1H, *J* = 8.3 Hz), 6.55 (s, 1H), 6.54 (s, 1H), 5.4 (apparent s, 0.4H), 5.36 (d, 0.5H, *J* = 1.3 Hz), 5.0 (apparent s, 0.5), 4.85 (apparent s, 0.5H), 4.38 (m, 1H), 3.83 (s, 1.2H), 3.81 (s, 1.7H), 3.76 (s, 3H), 3.71 (s, 6H), 3.6-3.38 (m, 2H), 2.3-1.9 (m, 4H), 1.51 (s, 6H), 1.46 (s, 3H).

¹³C NMR (100 MHz, CDCl₃): δ 172.4, 172.2, 161.4, 161.2, 160.3, 159.9, 154.5, 153.5, 153.5, 153.4, 134.9, 134.8, 132.9, 130.9, 128.8, 127.8, 127.7, 127.6, 127.0, 127.0, 114.6, 114.4, 95.4, 95.1, 82.8, 82.7, 80.2, 80.2, 63.6, 62.0, 58.6, 58.5, 56.0, 55.4, 55.3, 46.7, 46.4, 31.0, 29.9, 28.4, 28.4, 24.5, 23.7, 23.6

IR ν_{max}(ATR): 1756 cm⁻¹ (β-lactam C=O), 1697 cm⁻¹ (*N*-Boc-L-proline C=O)

HRMS: APCI calculated for $C_{29}H_{37}N_2O_9$ [$M + H^+$], 557.248291; found 557.24937, error -1.9 ppm. **ESI** calculated for $C_{29}H_{36}N_2NaO_9$ [$M + Na^+$], 579.23239; found 579.2313, error + 1.9 ppm

Purity RP-HPLC: 78 %

1-(*Tert*-Butyl) 2-((2*R*,3*R*)-2-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (108b)

108b was resolved from **108** using LC with a gradient of *n*-hexane: MTBE

Yield: 350mg (0.63mmol) 37%

Appearance: white amorphous powder

Melting Point: 62-65 °C

R_f: 0.33 (1:2 *n*-hexane:MTBE), *plate developed twice*

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 7.31 (apparent t, 2H, *J*= 8 Hz), 6.94 (apparent t, 2H, *J*= 8.5 Hz), 6.5 (s, 2H), 5.45 (d, 0.5H, *J*= 1.5 Hz), 5.34 (d, 0.4 H, *J*= 1.3 Hz), 4.93 (apparent s, 0.5H), 4.86 (apparent s, 0.5H), 4.39 (m, 1H), 3.83 (s, 1.5H), 3.82 (s, 1.5H), 3.79 (s,3H), 3.72 (s, 3H), 3.71 (s, 3H), 3.61-3.42 (m, 2H), 2.4-2.2 (m, 2H), 2.1-1.9 (m, 2H), 1.44 (s, 9H)

¹³C NMR (100 MHz, CDCl₃): δ 172.0, 171.2, 161.5, 161.2, 160.3, 160.1, 154.5, 154.5, 153.6, 153.5, 153.4, δ 135.0, δ 134.9, 133.0, 132.9, 128.8, 127.7, 127.0, 126.8, 126.5, 114.6, 114.5, 95.3, 95.4, 82.8, 82.7, 80.4, 80.1, 72.9, 63.6, 63.4, 63.6, 60.9, 59.1, 58.3, 56.0, 55.9, 55.3, 55.3, 49.4, 46.6, 46.3, 31.6, 31.0, 29.8, 28.2, 28.4, 28.3, 26.9, 24.7, 23.0

IR ν_{max}(ATR): 1757 cm⁻¹(β-lactam C=O), 1694 cm⁻¹ (*N*-Boc-L-proline C=O)

HRMS: APCI calculated for $C_{29}H_{37}N_2O_9$ [$M + H^+$], 557.248816; found 557.249357. error -1.0 ppm. **ESI** calculated for $C_{29}H_{36}N_2NaO_9$ [$M + Na^+$], 579.2324; found 579.231301, error -1.2 ppm

Purity (HPLC): 82%

2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl (*tert*-butoxycarbonyl)-L-phenylalaninate (109)

109 was synthesised from **109** and *N*-Boc-L-phenylalanine using **General Method XII**.

Yield: 363 mg (0.6 mmol) 60%

Appearance: white fluffy powder

R_f: 0.5 (60:40; *n*-hexane: ethyl acetate)

Melting point: 72 °C.

¹H NMR (400 MHz, CDCl₃): δ 7.34-7.2 (m, 7H), 6.94 (d, 2H, *J* = 8.3 Hz), 6.6 (s, 0.2H), 6.56 (s, 0.8H), 6.53 (s, 1H), 5.4 (apparent s, 0.5H), 5.33 (apparent s, 0.5H), 5 (m, 1H), 4.87 (apparent singlet 0.2H), 4.7 (m, 1.6H), 3.83 (s, 3H), 3.8 (s, 3H), 3.73 (s, 6H), 3.18 (m, 2H), 1.45 (s, 3H), 1.43 (s, 6H).

¹³C NMR (100 MHz, CDCl₃): δ 171.1, 160.5, 153.6, 153.1, 129.6, 128.7, 127.5, 127.3, 127.0, 126.7, 126.7, 114.6, 95.5, 83.0, 82.8, 80.3, 68.2, 63.5, 63.3, 61.0, 56.1, 55.4, 28.2

¹H NMR (600 MHz, DMSO- *d*₆): 7.5 (d, 0.5H, *J* = 4.6 Hz), 7.48 (d, 0.5H, *J* = 4.6 Hz), 7.42 (d, 1H, *J* = 8.6 Hz), 7.39 (d, 1H, *J* = 8.6 Hz), 7.3-7.19 (m, 5H), 6.97 (d, 2H, *J* = 8.6 Hz), 6.54 (s, 1H), 6.5 (s, 1H), 5.51 (d, 0.5, *J* = 1.6 Hz), 4.98 (d, 0.5, *J* = 1.6 Hz), 5.13 (apparent s, 0.5H), 4.87 (apparent s 0.49 H), 4.3 (m, 1H), 3.75 (s, 3H), 3.64 (s, 6H), 3.58 (s, 3H), 3.09-2.93 (m, 2H), 1.34 (s, 4.5H), 1.33 (s, 3H)

¹³C NMR (100 MHz, DMSO- *d*₆): δ 171.9, 171.5, 161.6, 160.1, 156.0, 153.6, 137.8, 137.6, 134.8, 132.8, 132.7, 129.8, 129.0, 127.6, 127.1, 127.0, 114.7, 96.0, 95.9, 82.3, 79.0, 62.6, 60.5, 60.2, 56.3, 55.6, 55.5, 36.7, 36.6, 28.6, 21.2

IR ν_{max}(ATR): 1756 cm⁻¹ (β-lactam C=O), 1709 cm⁻¹ (*N*-Boc-L-phenylalanine C=O)

HRMS: ESI calculated for C₃₃H₃₈N₂NaO₉ [**M** + Na⁺], 629.246952; found 629.247215, error + 0.4 ppm

Purity RP-HPLC: > 99%

2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl (((9*H*-fluoren-9-yl)methoxy) carbonyl)-L-phenylalaninate (110)

110 was synthesised from **17** and fmoc-L-phenylalanine using **General Method XII**.

Yield: 226 mg (0.31 mmol) 31%

Appearance: white fluffy powder

R_f: 0.53 (60:40; *n*-hexane: ethyl acetate)

Melting point: 77 °C.

¹H NMR (400 MHz, CDCl₃): δ 7.7 (t, 2H, *J* = 6.6 Hz), 7.6 (t, 2H, *J* = 6.6 Hz), 7.41 (m, 2H), 7.32 (m, 5H), 7.2 (m, 2H), 6.93 (apparent d, 2H, *J* = 8.8 Hz), 6.54 (s, 0.7H), 6.49 (s, 1.3H), 5.44 (apparent s, 0.4H), 5.34 (apparent s, 0.4H), 5.3 (apparent m, 0.4H), 5.23 (apparent m, 0.4H), 4.87 (apparent s, 0.6H), 4.81 (m, 1H), 4.66 (apparent s, 0.5H), 4.47-4.35 (m, 2H), 4.23 35 (m, 1H), 3.83 (s, 2H), 3.82 (s, 1H), 3.8 (s, 3H), 3.72 (s, 3.5H), 3.71 (s, 2.5H), 3.23 (m, 2H)

¹³C NMR (100 MHz, CDCl₃): δ 171.8, 168.5, 167.8, 167.0, 164.0, 160.3, 153.5, 141.2, 139.4, 132.9, 129.5, 128.9, 128.0, 127.0, 125.3, 120.0, 114.6, 112.4, 95.4, 66.0, 61.1, 56.0, 55.2, 47.1

¹H NMR (400 MHz, DMSO-*d*₆): δ 8.06 (m, 1H, NH), 7.86 (d, 2H, *J* = 8 Hz), 7.63 (d, 2H, *J* = 8 Hz), 7.39 (m, 4H), 7.29 (m, 5H), 7.23 (m, 1H), 6.95 (d, 2H, *J* = 8 Hz), 6.49 (s, 1H), 6.49 (s, 1H), 5.53 (apparent s, 0.6H), 5.5 (apparent s, 0.3H), 5.2 (apparent s, 0.5H), 4.9 (apparent s, 0.5H), 4.4 (m, 1H), 4.31-4.17 (m, 3H), 3.75 (s, 3H), 3.61 (s, 3H), 3.60 (s, 2H), 3.57 (s, 3H), 3.56 (s, 1H), 3.14 (apparent dd, 1H, *J* = 5 Hz, *J* = 14 Hz), 3.00 (m, 1H)

¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.5, 171.16, 170.8, 161.5, 160.0, 156.4, 153.6, 144.1, 141.2, 137.9, 137.7, 134.8, 132.8, 129.8, 129.6, 128.8, 128.0, 127.5, 127.1, 127.0, 125.6, 121.8, 120.6, 114.7, 95.9, 82.4, 82.3, 66.2, 62.5, 60.5, 60.2, 56.2, 55.7, 55.6, 47.0, 31.2, 31.0

¹⁵N NMR (60.8 MHz, DMSO-*d*₆): δ 88.5 (NH)

IR ν_{max} (ATR): 1756 cm⁻¹ (β-lactam C=O), 1709 cm⁻¹ (C=O)

HRMS: ESI calculated for C₄₃H₄₀N₂NaO₉ [**M** + Na⁺], 751.262602; found 751.264471, error - 2.5 ppm

Purity RP-HPLC: 89 %

2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl (*tert*-butoxycarbonyl)-L-tryptophanate (111**)**

111 was synthesised from **17** and *N*-Boc-L-tryptophan using **General Method XII**.

Yield: 250 mg (0.38 mmol) 59%

Appearance: yellow powder

R_f: 0.35 (1:1 *n*-hexane: ethyl acetate).

Melting Point: 87-92 °C

¹H NMR (600 MHz, CDCl₃): δ 8.22 (bs, 0.5H), 8.2 (bs, 0.5H), 7.63 (d, 0.5H, *J* = 8.6 Hz), 7.6 (d, 0.5H, *J* = 8.6 Hz), 7.38 (s, 0.5H), 7.36 (s, 0.5H), 7.24 (d, *J* = 8.6 Hz), 7.22 – 7.14 (m, 3H), 7.1 – 7.0 (m, 2H), 6.91 (d, 1H, *J* = 8.6 Hz), 6.88 (d, 1H, *J* = 8.6 Hz), 6.54 (s, 1H), 6.45 (s, 1H), 5.4 (apparent s, 0.6H), 5.23 (apparent s, 0.5H), 5.2 (apparent d, 0.4H *J* = 7.5 Hz, α NH), 5.1 (d, 0.5H, *J* = 7.5 Hz, α NH), 4.78 (m, 1H), 4.36 (apparent s, 0.5H), 3.8 (s, 1H), 3.8 (s, 1.5H), 3.79 (s, 1H), 3.79 (s, 2H), 3.72 (s, 3H), 3.71 (s, 3H), 3.38 (m, 2H), 1.46 (s, 4.5H), 1.44 (s, 4.5H)

¹³C NMR (100 MHz, CDCl₃): δ 171.2, 171.0, 161.3, 161.0, 160.2, 153.3, 153.4, 136.1, 136.0, 135.1, 135.0, 132.9, 127.7, 127.6, 126.9, 126.8, 123.3, 122.3, 119.8, 119.8, 118.8, 118.7, 114.6, 114.5, 111.3, 95.4, 83.1, 82.6, 80.2, 60.4, 56.0, 56.0, 55.3, 54.5, 54.2, 28.3, 27.7, 21.1, 14.2

¹⁵N NMR (60.8 MHz, CDCl₃): δ 123.6, 123.5, 86.8, 86.3

¹H NMR (400 MHz, DMSO-*d*₆): δ 10.95 (s, 0.5H), 10.91 (s, 0.5H, NH), 7.54 (d, 0.5H, *J* = 7.8 Hz), 7.52 (d, 0.5H, *J* = 7.8 Hz), 7.47 (d, 0.5H, *J* = 7.2 Hz), 7.42 (d, 0.5H, *J* = 7.2 Hz), 7.34 (m, 2H), 7.24 (m, 2H), 7.07 (m, 1H), 7.02-6.97 (m, 1H), 6.95 (d, 1H, *J* = 8 Hz), 6.93 (d, 1H, *J* = 8 Hz), 6.53 (s, 1H), 6.43 (s, 1H), 5.47 (d, 0.5H, *J* = 1.8 Hz), 5.42 (apparent s, 0.4H), 5.03 (apparent s, 0.5H), 4.5 (apparent s, 0.4H), 4.33 (m, 1H), 3.75 (s, 1H), 3.74 (s, 2H), 3.64 (s, 6H), 3.58 (s, 3H), 3.2-3.09 (m, 2H), 1.36 (s, 4.5H), 1.34 (s, 4.5H)

¹³C NMR (100 MHz, DMSO-*d*₆): δ 172.1, 171.7, 170.7, 161.7, 160.0, 155.9, 153.6, 136.0, 136.5, 134.8, 132.8, 132.7, 128.9, 128.8, 124.6, 124.4, 121.5, 119.0, 118.9, 118.6, 118.5, 114.7, 111.9, 109.8, 109.6, 100.3, 96.0, 82.2, 82.0, 79.0, 62.6, 60.5, 60.2, 56.2, 55.6, 55.1, 28.6, 27.0, 21.2

¹⁵N NMR (60.8 MHz, DMSO-*d*₆): δ 134.9, 91.4, 89.9

IR ν_{max} (ATR): 1730 cm⁻¹ (tryptophan C=O), 1757 (β-lactam C=O) cm⁻¹

HRMS: APCI calculated for C₃₅H₃₈N₃O₉ [**M** - H⁺], 644.261353; found 644.261945, error + 0.6 ppm

Purity RP-HPLC: >99%

2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl (*tert*-butoxycarbonyl)-L-tryptophanate (111a**)**

111a was characterised in CDCl₃ at 600 MHz from **111** but was not resolved.

¹H NMR (600 MHz, CDCl₃): δ 8.20 (bs, 1H), 7.64 (d, 1H, *J* = 7.8 Hz), 7.36 (d, 1H, *J* = 8 Hz), 7.24 (d, 2H, *J* = 8 Hz), 7.2 (apparent m, 1H), 7.19 (apparent m, 1H), 7.15 (apparent m, 1H), 6.91 (d, 2H, *J* = 8 Hz), 6.54 (s, 2H), 5.41 (apparent s, 1H), 5.1 (d, 1H, *J* = 7.7 Hz, α-NH), 4.8 (apparent s, 1H), 4.77 (m, 1H), 3.81 (s, 3H), 3.79 (s, 3H), 3.72 (s, 6H), 3.39–3.36 (2H), 1.46 (s, 9H).

¹³C NMR (100 MHz, CDCl₃): δ 171.1, 161.3, 160.2, 155.3, 153.4, 136.1, 135.1, 127.7, 126.8, 123.3, 122.2, 119.8, 118.8, 111.3, 95.3, 82.6, 80.1, 63.3, 60.95, 56.04, 55.34, 54.5, 28.32, 27.69

¹⁵N NMR (60.8 MHz, CDCl₃): δ 123.5 (Tryptophan ring NH), 86.3 (α-NH)

2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl (*tert*-butoxycarbonyl)-L-tryptophanate (111b**)**

111b was characterised in CDCl₃ at 600 MHz from **111** but was not resolved.

¹H NMR (600 MHz, CDCl₃): δ 8.22 (s, 1H, NH Tryptophan ring), 7.62 (d, 1H, *J* = 7.8 Hz), 7.36 (d, 1H, *J* = 8.5 Hz), 7.15 (m, 1H), 7.14 (m, 1H), 7.06 (d, 8 Hz), 7.03 (t, 1H, *J* = 7.3 Hz), 6.87 (d, 2H, *J* = 8 Hz), 6.53 (s, 1H), 6.5 (s, 1H), 5.22 (apparent s, 1H), 5.18 (d, 1H, *J* = 7.3 Hz, α-NH), 4.79 (m, 1H), 4.37 (apparent s, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.71 (s, 6H), 3.41–3.34 (m, 2H), 1.44 (s, 9H)

¹³C NMR (100 MHz, CDCl₃): δ 171.2, 161.1, 160.1, 155.2, 153.5, 136.0, 135.0, 127.7, 127.6, 126.9, 123.1, 119.8, 118.7, 114.5, 111.3, 109.7, 95.4, 83.1, 80.1, 63.0, 61.0, 56.0, 55.3, 54.3, 28.3, 27.6

¹⁵N NMR (60.8 MHz, CDCl₃): δ 123.6 (Tryptophan ring NH), 86.8 (α-NH)

2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl (*tert*-butoxycarbonyl)-L-valinate (112**)**

112 was synthesised from **17** and *N*-Boc-L-valine using **General Method XII**.

Yield: 329 mg (0.58 mmol) 58%

Appearance: white solid.

R_f: 0.38 (20:80; *n*-hexane: MTBE)

Melting point: 78–83 °C

¹H NMR (400 MHz, CDCl₃): δ 7.32 (d, 1H, *J* = 8.3 Hz), 7.29 (d, 1H, *J* = 8.3 Hz), 6.95 (d, 1H, *J* = 8.6 Hz), 6.94 (d, 1H, *J* = 8.6 Hz), 6.56 (s, 1H), 6.55 (s, 1H), 5.45 (d, 0.6 H, *J* = 1.8 Hz), 5.35 (apparent s, 0.3H), 5.03 (apparent m, 1, NH), 4.95 (apparent s, 0.6H), 4.88 (apparent s, 0.5H), 4.34 (m, 1H), 3.84 (s, 1.5H), 3.83 (s, 1.5H), 3.79 (s, 1.5H), 3.78 (s, 1.5H), 3.73 (s, 3H), 3.72 (s, 3H), 2.23 (m, 1H), 1.48 (s, 6H), 1.46 (s, 3H), 1.04 (d, 1.5H, *J* = 6.6 Hz), 1.03 (s, 1.5H, *J* = 6.6 Hz), 0.98 (d, 1.5H, *J* = 6.6 Hz), 0.96 (d, 1.5 H, *J* = 6.6 Hz)

¹³C NMR (100 MHz, CDCl₃): δ 171.4, 161.2, 160.2, 155.8, 134.8, 131.0, 128.8, 127.8, 126.7, 114.5, 95.3, 83.0, 82.5, 80.1, 65.7, 63.3, 61.06, 58.8, 58.5, 56.1, 55.3, 30.9, 28.2, 19.1, 17.9, 17.4, 15.3

¹H NMR (400 MHz, DMSO-*d*₆): δ 7.42 (m, 1H), 7.38 (m, 1H), 6.55 (s, 1.3H), 6.54 (s, 0.7H), 5.55 (apparent m, 1H), 5.89 (apparent s, 0.6H), 5.12 (apparent s, 0.4H), 3.95 (m, 1H), 3.73 (s, 3H), 3.63 (s, 6H), 3.57 (s, 6H), 2.1 (m, 1H), 1.40 (s, 4.5H), 1.38 (s, 4.5H), 0.94 (m, 6H).

¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.8, 171.6, 162.0, 153.7, 134.8, 132.9, 129.0, 127.7, 114.7, 96.0, 79.0, 62.5, 60.6, 59.8, 56.3, 55.6, 38.7, 30.1, 29.9, 28.7, 28.6, 19.5, 19.4, 19.1, 18.9

IR ν_{max}(ATR): 1757 (C=O) cm⁻¹

HRMS: ESI calculated for C₂₉H₃₈N₂O₉ [**M** + Na⁺], 581.246952; found 581.247950, error -1.7 ppm

Purity RP-HPLC: 97%

2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl (2-(9*H*-fluoren-9-yl)acetyl)- L -valinate (113)

113 was synthesised from **17** and fmoc-L-valine using **General Method XII**.

Yield: 370 mg, (0.54 mmol) 54%

Appearance: white solid.

R_f: 0.38 (20:80; *n*-hexane: MTBE)

Melting point: 78-83 °C

¹H NMR (400 MHz, CDCl₃): δ 7.78 (t, 2H, *J* = 7.6 Hz), 7.642 (m, 2H, *J* = 7.6 Hz), 7.41 (complex m, 2H), 7.32 (m, 5H), 6.93 (apparent triplet, 2H, *J* = 8 Hz), 6.55 (s, 0.91H), 6.52 (s, 1.1), 5.47 (apparent s, 0.4H), 5.38 (d, 0.4H, *J* = 1.8 Hz), 5.30 (apparent s, 0.3H), 5.29 (apparent s, 0.4H), 4.94 (apparent s, 0.5H), 4.88 (apparent s, 0.5H), 4.49-4.36 (m, 3H), 4.25 (m, 1H), 3.83 (s, 1.5H), 3.82 (s, 1.5H), 3.79 (s, 1.5H), 3.78 (s, 1.5H), 3.72 (s, 3H), 3.69 (s, 3H), 2.3 (m, 1H), 1.0-0.98 (m, 6H)

¹³C NMR (100 MHz, CDCl₃): δ 176.9, 175.9, 161.5, 153.4, 143.9, 141.4, 133.0, 129.9, 127.7, 127.1, 120.0, 114.7, 95.5, 95.2, 67.1, 61.1, 60.4, 56.1, 55.4, 47.0, 30.9, 21.0, 19.1, 17.9, 14.2

¹H NMR (400 MHz, DMSO-*d*₆): δ 7.69 (d, 1H, NH, *J* = 7.7 Hz), 7.83 (m, 2H), 7.73 (m, 1H), 7.41 (m, 3H), 7.32 (m, 2H), 6.94 (d, 2H), 6.54 (s, 1H), 6.51 (s, 1H), 5.59 (d, 0.5H, *J* = 1.8 Hz), 5.55 (d, 0.5H, *J* = 1.8 Hz), 5.21 (d, 0.5H, *J* = 1.8 Hz), 5.14 (d, 0.5H, *J* = 1.8 Hz), 4.31 (m, 1H), 4.24 (m, 1H), 4.08 (m, 1H), 3.74 (s, 3H), 3.62 (s, 3H), 3.60 (s, 3H), 3.57 (s, 3H), 2.15 (s, 1H), 0.96 (m, 6H).

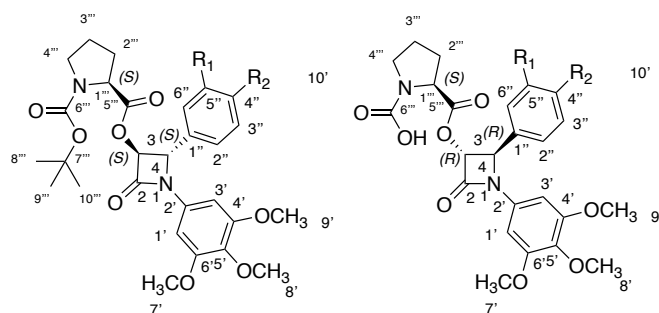
¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.5, 167.7, 160.3, 144.4, 141.3, 132.6, 129.4, 129.0, 128.0, 127.6, 125.8, 121.9, 120.7, 114.9, 96.0, 82.1, 66.5, 65.4, 62.5, 60.6, 60.3, 60.0, 56.3, 55.7, 47.1, 30.3, 21.8, 21.3

IR ν_{max} (ATR): 1730 cm⁻¹ (valine C=O), 1757 cm⁻¹ (β-lactam C=O)

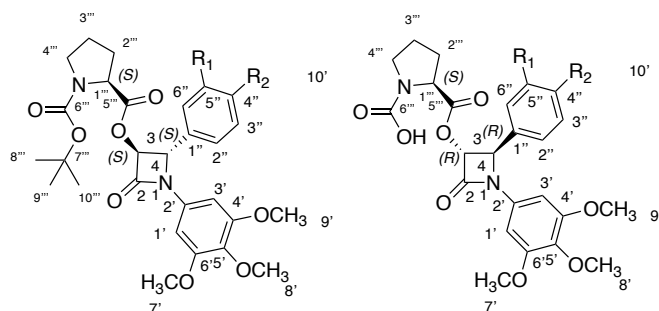
HRMS: ESI calculated for C₃₉H₄₀N₂NaO₉ [**M** + Na⁺], 703.262602; found 703.264394, error + 2.5 ppm

Purity RP-HPLC: >90%

Numbering nomenclature for 3- hydroxy *N*-Boc-L-proline derivatised diastereomers.



Diastereomer code	3X	4X	R ₁	R ₂
114a	Diastereomer mixture		F	OCH ₃
114a	<i>S</i>	<i>S</i>	F	OCH ₃
114b	<i>R</i>	<i>R</i>	F	OCH ₃
115	Diastereomer mixture		OTBDMS	OCH ₃
116	Diastereomer mixture		OH	OCH ₃
116a	<i>S</i>	<i>S</i>	OH	OCH ₃



Diastereomer code	3X	4X	R ₁	R ₂
116b	<i>R</i>	<i>R</i>	OH	OCH ₃
117	Diastereomer mixture		Cl	OCH ₃
117a	<i>S</i>	<i>S</i>	Cl	OCH ₃
117b	<i>R</i>	<i>R</i>	Cl	OCH ₃
118	Diastereomer mixture		CH ₃	OCH ₃
118a	<i>S</i>	<i>S</i>	CH ₃	OCH ₃
118b	<i>R</i>	<i>R</i>	CH ₃	OCH ₃
119	Diastereomer mixture		H	SCH ₃
119a	<i>S</i>	<i>S</i>	H	SCH ₃
119b	<i>R</i>	<i>R</i>	H	SCH ₃
120	Diastereomer mixture		Br	OCH ₃
120a	<i>S</i>	<i>S</i>	Br	OCH ₃
120b	<i>R</i>	<i>R</i>	Br	OCH ₃
121	Diastereomer mixture		H	F
121a	<i>S</i>	<i>S</i>	H	F
121b	<i>R</i>	<i>R</i>	H	F
122	Diastereomer mixture		NO ₂	NO ₂
122a	<i>S</i>	<i>S</i>	NO ₂	NO ₂
122b	<i>R</i>	<i>R</i>	NO ₂	NO ₂

1-(*Tert*-butyl) 2-(-2-(3-fluoro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (114**)**

114 was synthesised from **18** and *N*-(Boc)-L-proline using **General Method XII**.

Yield: 1.04g (1.81 mmol) 80%

¹H NMR (400 MHz, CDCl₃): δ (diastereomers and rotamers present) 7.11 (m, 2H), 7.00 (m, 1H), 6.55 (3, 0.7H), 6.54 (s, 0.7H), 6.53 (s, 0.6H), 5.40 (d, 0.2H, *J* = 1.8 Hz), 5.36 (d, 0.2H, *J* = 1.8 Hz), 5.34 (d, 0.3H, *J* = 1.8 Hz), 5.31 (d, 0.3H, *J* = 1.8 Hz), 4.99 (apparent s, 0.4H), 4.92 (apparent s, 0.3H), 4.83 (apparent s, 0.5H), 4.39 (m, 1H), 3.94 (s, 1H), 3.91 (s, 1H), 3.90 (s, 1H), 3.81 (s, 1H), 3.80 (s, 2H), 3.75 (s, 5H), 3.74 (s, 2H), 3.58- 3.41 (m, 2H), 2.33 – 2.18 (m, 2H), 2.10- 1.89 (m, 2H), 1.53 (s, 2H), 1.47 (m, 2H), 1.43 (s, 6H)

¹⁹F NMR (376 MHz, CDCl₃): δ -132.9, -133.3, -133.4, -133.5

IR ν_{max}(ATR): 1757 (β-lactam C=O) cm⁻¹, 1691 cm⁻¹ (*N*-Boc-L-proline C=O)

1-(*Tert*-butyl) 2-((2*S*,3*S*)-2-(3-fluoro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (114a**)**

114a was resolved from **114** using LC as described in **General Method XII**.

Yield resolved from diastereomer mixture: 472mg (0.82 mmol) 45%

Appearance: white amorphous powder

Melting Point: 78 °C

R_f: 0.36 - 0.39 (2:1 MTBE: *n*-hexane): plate developed twice

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 7.25 (d, 1H, *J*=7.5 Hz, H_{2''&6''}), 7.18 (d, 1H, *J*=7.5 Hz, H_{3''&5''}), 7.09 (t, 1H, *J*=8 Hz, H_{3''}, rotamer 1), 6.95 (t, 1H, *J*=8 Hz, H_{3''}, rotamer 2), 6.54 (s, 2H, H_{1'&3'}), 5.36 (s, 0.5 H), 5.34 (s, 0.5H, rotamer 2), 4.99, (s, 0.5H, rotamer 2), 4.83 (s, 0.5H, rotamer 2), 4.38 (m, 1H, H_{1'''}), 3.91 (s, 1.5H, H_{8'}, rotamer 1), 3.89 (s, 1.5H, H_{8'}, rotamer 2), 3.79 (s, 3H, H_{10'}), 3.73 (s, 6H, H_{7'&9'}), 3.50 (m, 2H, proline CH₂), 2.27 (m, 2H, proline CH₂), 1.99 (m, 2H, proline CH₂), 1.50 (s, 4.5H, , *t*-butyl, H_{8'''-10'''}), 1.47 (s, 9H, rotamer 2, H_{8'''-10'''})

¹³C NMR (100 MHz, CDCl₃): δ 161.5, 154.1, 154.6, 153.2, 152.7, 152.7, 151.1, 148.0 (m), 134.9, 132.6, 128.4 (m), 124.4 (d, *J*= 25 Hz), 115.3 (d, *J*= 25 Hz), 114.5 (d, *J*=7 Hz), 96.1 (d, *J*= 15 Hz), 82.1 (d, *J*= 13 Hz), 79.7 (d, 5.3 Hz), 62.1, 61.9, 60.6, 58.8 (d, *J*= 9 Hz), 56.5, 56.3, 46.9, 46.7, 38.7, 30.8, 29.8, 28.5, 28.4, 24.5, 23.7

¹⁹F NMR (376 MHz, CDCl₃): δ -132.9, -133.2, -133.5

IR ν_{max}(ATR): 1756 (β-lactam C=O) cm⁻¹ 1690 cm⁻¹ (*N*-Boc-L-proline C=O).

HRMS: APCI calculated for C₂₉H₃₅FN₂O₉ [**M + H**⁺], 575.238645; found 575.239935, error + 2.2 ppm **ESI** calculated for C₂₉H₃₅FN₂NaO₉ [**M + Na**⁺], 597.221775; found 597.221880, error + 0.2 ppm

Purity (RP-HPLC): 86 %

1-(*Tert*-butyl) 2-((2*R*,3*R*)-2-(3-fluoro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (114b**)**

114b was resolved from **114** using LC described in **General Method XII**.

Yield resolved from diastereomer mixture: 250mg (0.44 mmol) 24%

Appearance: white powder

Melting Point: 71°C

R_f: 0.31-0.34 (2:1; MTBE: *n*-hexane), plate developed twice

¹H NMR (400 MHz, CDCl₃): δ 7.12 (t, 2H, *J*=7 Hz), 7.18 (m, 1H, *J*=7.9 Hz), 6.53 (s, 1H, rotamer 1), 6.52 (s, 2H, rotamer 2), 5.38 (apparent s, 0.5H, H₃, rotamer 1), 5.31 (s, 0.5H, H₃, rotamer 2), 4.91 (s, 0.5H, H₄, rotamer 1), 4.82 (s, 0.5H, H₄, rotamer 2), 4.39 (m, 1H, H_{1'''}), 3.92 (s, 1.5H, rotamer 1), 3.9 (s, 1.5H, rotamer 2), 3.79 (s, 3H), 3.74 (s, 3H, rotamer 1), 3.7 (s, 3H, rotamer 2), 3.50 (m, 2H, proline CH₂), 2.20 (m, 2H, proline CH₂), 2.05 (m, 2H, proline CH₂), 1.40 (s, 9H, H_{8'''-10'''})

¹³C NMR (100 MHz, CDCl₃): δ 172.5, 172.3, 161.2, 160.2, 154.6, 153.8, 53.3, 153.7, 153.6, 151.4, 151.3, 148.2, 148.0, 135.3, 135.2, 132.7, 129.9, 128.3, 128.0, δ127.7, 127.6, 126.5, δ126.2, δ125.3, 122.4, 22.4, δ117.7, 114.4, 114.3, 113.9, 113.7, 95.5, 95.3, 82.8, 82.6, 82.5, 80.4, 80.2, 64.1, 63.1, 60.9, 58.7, 58.6, 56.3, 56.2, 46.8, 46.4, 31.0, 30.8, 29.9, 28.5, 28.3, 24.5, 23.6

¹⁹F NMR (376 MHz, CDCl₃): δ -132.9, -133.11, -133.4, -133.5

IR ν_{max}(ATR): 1757 cm⁻¹ (β-lactam C=O), 1694 cm⁻¹ (*N*-Boc-L-proline C=O).

HRMS: APCI calculated for C₂₉H₃₅FN₂O₉ [**M⁺ + H**], 575.238613; found 575.239935, error -2.3 ppm.

ESI calculated for C₂₉H₃₅FN₂NaO₉ [**M + Na**⁺], 597.222267; found 597.22188, error -0.6 ppm

Purity (HPLC): 82%

1-(*Tert*-butyl) 2-(2-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl) azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (115**)**

115 was synthesised from **67a** and *N*-Boc-L-proline using **General Method XII**.

Yield: 58%

R_f: 0.49 (2:1 MTBE:*n*-hexane), *plate developed once*

¹H NMR (400 MHz, CDCl₃): δ (diastereomers and rotamers present) 6.78 (complex m, 3H), 6.52 (s, 1H), 6.51 (s, 1H), 5.39 (d, *J*=1.3 Hz, 0.2H), 5.36 (apparent s, 0.18H), 5.34 (d, 0.18H, *J*=1.3 Hz), 5.32 (d, 0.24H, *J*=1.3 Hz), 5.3 (apparent s, 0.19H), 4.9 (apparent s, 0.2H), 4.8 (apparent s, 0.19H), 4.79 (d, *J*=1.3 Hz, 0.18H), 4.77 (apparent s, 0.4H) 4.35 (complex m, 1H, H₁...), 3.79, 3.78, 3.77 (3 X s, 3H), 3.74 & 3.72 (2 X s, 3H), 3.68 (s, 6H), 3.42 (m, 2H), 2.27 (m, 2H), 1.90 (m, 2H), 1.42 & 1.41 (2 X s, 3H, *t*-butyl (C(CH₃)₃)), 0.92 & 0.89 (2 X s, 3H, (C(CH₃)₃)), 0.06 & 0.05 (2 X s, 1.5H, CH₃ of TBDMS), 0.04 & 0.03 (2 X S, 1.5H, CH₃ of TBDMS)

¹³C NMR (100 MHz, CDCl₃): δ 172.1, 172.0, 171.9, 171.8, 170.6, 169.5, 167.7, 161.5, 161.4, 161.2, 161.1, 154.5, 153.6, 153.5, 153.4, 152.4, 151.7, 151.6, 151.4, 151.4, 151.5, 151.4, 150.8, 145.7, 145.6, 145.5, 145.4, 144.6, 138.2, 135.0, 134.9, 134.8, 133.0, 132.9, 132.9, 132.9, 130.9, 130.4, 130.4, 130.3, 127.4, 127.3, 127.1, 120.19, 120.1, 120.0, 119.9, 119.4, 118.9, 118.8, 118.8, 112.4, 112.3, 111.3, 107.6, 95.5, 95.4, 95.3, 83.2, 82.6, 82.5, 82.3, 80.3, 80.2, 80.1, 80.0, 79.9, 63.8, 63.8, 63.5, 63.5, 63.3, 63.1, 61.6, 60.9, 60.8, 59.0, 58.9, 58.8, 58.6, 58.4, 56.0, 56.0, 55.9, 55.8, 55.7, 55.5, 55.4, 46.60, 46.3, 46.3, 31.9, 31.0, 30.8, 29.8, 29.6, 28.4, 28.5, 28.3, 28.2, 25.6, 25.6, 24.4, 23.6, 23.5, 23.5, 20.5, 20.4, 20.4, 18.4, 18.3, 15.1, -4.8, -4.9

1-(*Tert*-butyl) 2-(-(2-(3-hydroxy-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (116)

115 was deprotected using **General Method X** to yield a mixture of diastereomers **116**.

Yield for desilylation and purification: 82%

Total yield for diastereomer coupling: 42%

Appearance: Yellow/white gel

R_f: 0.75 (1:1 ethyl acetate: *n*-hexane), 0.49 (2:1 MTBE: *n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ 6.94-6.89 (m, 3H), 6.57 (s, 0.5H), 6.56 (s, 1H), 6.56 (s, 0.7H), 5.45 (s, 0.3H), 0.42 (s, 0.2H), 5.38 (s, 0.25H), 5.36 (s, 0.3H), 4.94 (s, 0.2H), 4.88 (s, 0.4H), 4.81 (s, 0.4H), 4.39 (m, 1H), 3.92 (s, 1.4H), 3.91 (s, 1H), 3.9 (s, 1H), 3.79 (s, 1H), 3.78 (s, 2H), 3.74 (s, 6H), 3.61-3.39 (m, 2H), 2.35-2.24 (m, 2H), 2.10-1.91 (m, 2H), 1.61 (s, 3H), 1.45 (s, 6H)

¹³C NMR (100 MHz, CDCl₃): δ 172.1, 172.0, 171.9, 171.8, 170.6, 169.5, 167.7, 161.5, 161.4, 161.2, 161.1, 154.5, 153.6, 153.5, 153.4, 152.4, 151.7, 151.6, 151.4, 151.4, 151.5, 151.4, 150.8, 145.7, 145.6, 145.5, 145.4, 144.6, 138.2, 135.0, 134.9, 134.8, 133.0, 132.9, 132.9, 132.9, 130.9, 130.4, 130.4, 130.3, 127.4, 127.3, 127.1, 120.19, 120.1, 120.0, 119.9, 119.4, 118.9, 118.8, 118.8, 112.4, 112.3, 111.3, 107.6, 95.5, 95.4, 95.3, 83.2, 82.6, 82.5, 82.3, 80.3, 80.2, 80.1, 80.0, 79.9, 63.8, 63.8, 63.5, 63.5, 63.3, 63.1, 61.6, 60.9, 60.8, 59.0, 58.9, 58.8, 58.6, 58.4, 56.0, 56.0, 55.9, 55.8, 55.7, 55.5, 55.4, 46.60, 46.3, 46.3, 31.9, 31.0, 30.8, 29.8, 29.6, 28.4, 28.5, 28.3, 28.2, 25.6, 25.6, 24.4, 23.6, 23.5, 23.5, 20.5

1-(*Tert*-butyl) 2-((2*S*,3*S*)-2-(3-hydroxy-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (116a)

116a was resolved from **116** using LC as described in **General Method XII**.

Yield resolved from diastereomer mixture: 112 mg (0.2 mmol) 20%

Appearance: white powder

R_f: 0.26 (plate development 1), 0.56 (plate development 2), (3:1 MTBE : *n*-hexane), 0.42 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 6.93-6.84 (m, 3H), 6.57 (s, 2H), 5.42 (s, 0.45H), 5.38 (s, 0.48H), 4.95 (s, 0.6H), 4.81 (s, 0.4H), 4.38 (s, 1H), 3.92 (s, 2H), 3.90 (s, 1H), 3.78 (s, 3H), 3.74 (s, 6H), 3.61 – 3.40 (m, 3H), 2.30-2.21 (m, 1H), 2.1-1.9 (m, 3H), 1.52 (s, 6H), 1.47 (s, 3H)

¹H NMR (600 MHz, DMSO-*d*₆): δ (rotamers present) 9.12 (bs, 1H, OH), 9.09 (bs, 1H, OH), 6.94 (apparent t, 1H, *J* = 7.6 Hz, H_{3''}), 6.89 (d, 1H, *J* = 8.6 Hz, H_{3''}), 6.84 (d, 1H, *J* = 9 Hz, H_{2''}), 6.57 (s, 1H, H_{1'&3'}), 6.55 (s, 1H, H_{1'&3'}), 5.51, 5.47 (apparent s, 1H, H₃), 5.13, 5.10 (apparent s, 1H, H₄), 4.30 4.30 (m, 1H, H_{10''}), 3.76 (s, 3H, H_{10'}), 3.762 (s, 6H, H_{7'&9'}), 3.58 (s, 3H, H_{8'}), 2.30 (m, 2H), 1.95 (m, 2H), 1.86 (m, 2H, H_{1''}, H_{2''}, H_{3''}), 1.42, 1.37, 1.30 (s, 9H, H_{8''}, 10'')

¹³C NMR (100 MHz, DMSO-*d*₆): δ 172.3 (C_{5''}), 172.1 (C_{6''}), 161.6 (C₂), 154.1, 153.6, 153.2 (C_{4'&6'}), 148.7, 148.6 (C_{4''}), 147.7 (C_{5''}), 134.9 (C_{5'}), 132.58 (C_{2'}), 128.1, 128.01 (C_{1''}), 118.7, 118.6 (C_{6''}), 114.23 (C_{3''}), 112.7 (C_{2''}), 96.1, 95.9 (C_{1'&3'}), 82.3 (C₃), 72.3 (C_{7''}), 62.7 (C_{1''}), 60.53 (C_{8'}), 56.2 (C_{7'&9'}), 56.0 (C_{10'}), 46.8, 46.7, 28.3, 24.3, 23.6

IR ν_{max} (ATR): 3356 cm⁻¹ (OH), 1754 cm⁻¹ (β-lactam C=O), 1690 cm⁻¹ (*N*-Boc-L-proline C=O)
HRMS: APCI calculated for C₂₉H₃₇N₂O₁₀ [**M+H**⁺], 573.244453; found 573.244272, error -0.3 ppm

Purity (RP-HPLC): 88%

1-(*Tert*-butyl) 2-((2*R*,3*R*-2-(3-hydroxy-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (116b**)**

116b was resolved from **116** using LC as described in **General Method XII**.

Yield: 100 mg (0.17 mmol) 17%

Appearance: white powder

R_f: 0.26 (plate development 1), 0.54 [plate development 2 (3:1 MTBE :*n*-hexane)]

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 9.11 (bs, 1H, OH, H_{5'}), 6.94 (d, 1H, *J* = 8.1, H_{3''}), 6.89 (d, 1H, *J* = 8.1 Hz, H_{2''}), 6.85 (d, 1H, *J* = 2 Hz, H_{6''}), 6.55 (s, 1H, H_{1'&3'}), 6.54 (s, 1H, H_{1&3}), 5.51, 5.47 (apparent s, 1H, H₃), 5.13, 5.08 5.04 (apparent s, 1H, H₄), 4.31 (m, 1H, H_{1''}), 3.76 (s, 3H, H_{10'}), 3.64 (s, 6H, H_{7'&9'}), 3.59 (s, 3H, H_{8'}), 2.29 (m, 2H), 1.99 (m, 2H), 1.84 (m, 2H), 1.39 (s, 3H, H_{9''}), 1.34 (s, 6H, H_{8''&10''})

¹³C NMR (100 MHz, CDCl₃): δ 172.3 (C_{5''}), 172.3 (C_{5''}), 172.192 (C_{6''}), 161.47 (C₂), 154.07, 153.9, 153.3 (rotamers) (C_{5'&6'}), 48.6 (C_{4''}), 147.2 (C_{5''}), 134.9 (C_{5'}), 132.91, 132.83 (C_{2'}), 128.2, 128.1 (C_{1''}), 118.71, 118.57 (C_{2''}), 114.26, 114.34, 114.28 (C_{3''}), 112.7 (C_{6''}), 96.0 (C_{1'&3'}), 95.9 (C_{1'&3'}), 82.2 (C₃), 82.2 (C₃), 79.7, 79.7, 62.9, 62.6 (C₄), 62.5 (C₄), 60.5 (C_{10'}), 58.8, 56.3 (C_{7'&9'}), 56.1 (C_{7'&9'}), 28.5 (C_{7''}), 28.4 (C_{8''&10''}), 27.3, 24.5, 23.6 (C_{2''}, C_{3''}, C_{4''})

IR ν_{max} (ATR): 3356 cm⁻¹ (OH) 1754 cm⁻¹ (β-lactam C=O), 1687 cm⁻¹ (*N*-Boc-L-proline C=O)
HRMS: APCI calculated for C₂₉H₃₇N₂O₁₀ [**M+H**⁺], 573.242595; found 573.244272, error -2.9 ppm

Purity (RP-HPLC): 95%

1-(*Tert*-butyl) 2-(2-(3-chloro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (117**)**

117 was synthesised from **19** and *N*-Boc-L-proline using **General Method XII**.

Yield: 912 mg (1.54 mmol) 61%

Appearance: white amorphous solid

Melting Point: 77-82 °C

¹H NMR (400 MHz, CDCl₃): δ (rotamers and diastereomers present) 7.39 (dd, 1H, *J* = 2.3 Hz, *J* = 8.6 Hz, H_{2''}), 7.27- 7.21 (m, 1H, H_{6''}), 7.96 (apparent triplet, 1H, *J* = 8.0 Hz, H_{3''}), 6.54 (s, 0.4H, H_{1'&3'}), 6.53 (s, 0.6H, H_{1'&3'}), 6.52 (s, 0.8H, H_{1'&3'}), 5.4 (apparent s, 0.3H, H₃), 5.38 (apparent s, 0.1H, H₃), 5.34 (apparent s, 0.2H, H₃), 5.3 (apparent s, 0.4H, H₃), 4.97 (apparent s, 0.2H, H₄), 4.91 (apparent s, 0.4H, H₄), 4.81 (apparent s, 0.4H, H₄), 4.39 (m, H_{1''}), 3.92 (s, 1.5H, OCH₃, H_{8'}), 3.91 (s, 1H, H_{8'}), 3.90 (s, 0.5H, H_{8'}), 3.78 (s, 0.6H, OCH₃), 3.77 (s, 2.4H, H_{10'}), 3.74 (s, 3H, H_{7'&9'}), 3.73 (s, 3H, H_{7'&9'}), 3.6- 3.41 (m, 2H, proline CH₂), 2.35 – 1.89 (m, 4H, proline CH₂), 1.52 (s, 1.6H, *t*- butyl), 1.46 (s, 1.2H, *t*- butyl), 1.44 (s, 3H, *t*- butyl), 1.43 (s, 4H, *t*- butyl)

¹³C NMR (100 MHz, CDCl₃): 172.5, 172.1, 172.0, 161.2, 160.9, 155.7, 155.5, 154.6, 153.6, 153.5, 135.3, 135.1, 132.8, 128.4, 128.2, 127.9, 126.0, 123.4, 112.6, 112.4, 112.3, 95.5, 95.4, 95.3, 82.8, 82.6, 80.4, 80.2, 63.0, 62.8, 62.7, 61.0, 59.1, 58.8, 58.6, 56.2, 46.6, 46.3, 31.0, 29.8, 28.5, 28.4, 28.4, 27.0, 24.7, 24.6, 23.8, 23.7

IR ν_{max}(ATR): 1759 cm⁻¹ (β-lactam C=O), 1693 cm⁻¹ (*N*-Boc-L-proline C=O)

HRMS: ESI calculated for C₂₉H₃₅ClN₂NaO₉ [**M+Na**⁺], 613.192329; found 613.192975, error - 1.1 ppm

1-(*tert*-butyl) 2-((2*S*,3*S*)-2-(3-chloro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (117a)

117a was resolved from **117** using LC as described in **General Method XII**.

Yield resolved from diastereomer mixture: 339 mg (0.57mmol) 37%

Appearance: white amorphous solid

Melting Point: 75-82 °C

R_f: 0.26 (plate development 1), 0.46 (plate development 2), (2:1 MTBE :*n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 7.39 (s, 1H), 7.32 (dd, 1H, *J* = 8.7 Hz, *J* = 1.8 Hz), 6.98 (d, 0.4H, *J* = 8.7 Hz), 6.94 (d, 0.4H, *J* = 8.7 Hz), 5.39 (s, 0.4H), 5.35 (s, 0.6H), 4.98 (s, 0.7H), 4.82 (s, 0.4H), 4.39 (m, 1H), 3.93 (s, 1H), 3.91 (s, 2H), 3.83 (s, 2H), 3.81 (s, 3H), 3.74 (s, 6H), 3.63-3.41 (m, 2H), 2.36-2.19 (m, 1H), 2.11 – 1.91 (m, 3H), 1.53 (s, 6H), 1.48 (s, 3H)

¹H NMR (600 MHz, DMSO-*d*₆): δ 7.63 (d, 1H, H_{2'}, *J*=2.9 Hz, H_{6''}), 7.45 (dd, 1H, *J*=8 Hz, *J*=2.9 Hz, H_{2''}), 7.17 (t, 1H, *J*=8 Hz, H_{3''}), 6.57 (s, 1H, H_{1'&3'}), 6.49 (s, 1H, H_{1'&3'}), 5.62, 5.55 (apparent ss, H₃), 5.21, 5.19 (apparent ss, 1H, H₄), 4.30 (m, 1H, H_{1''}), 3.86 (s, 3H, H_{10'}), 3.65 (s, 6H, H_{7'&9'}), 3.59 (s, 3H, H_{8'}), 3.36 (m, 2H), 2.27 (m, 1H), 1.98 (m, H_{2''}), 1.86 (m, 2H, H_{3''}), 1.39 (s, 3H, H_{9''}), 1.36 (s, 6H, H_{8''&10''})

¹³C NMR (100 MHz, DMSO-*d*₆): δ 172.33 (C_{6''}), 172.21 (C_{5''}), 161.53 (C₂), 155.31, 155.24 (C_{4''}), 154.1 (C_{5''}), 153.6 (C_{1'&3'}), 153.1 (C_{1'&3'}), 134.9 (C_{5'}), 132.6 (C_{2'}), 129.4, 128.9, 128.8, (C_{2''}), 127.8, 127.6 (C_{3''}), 121.7, 121.6 (C_{1''}), 113.5, 113.4 (C_{6''}), 96.1 (C_{1'&3'}), 96.1 (C_{1'&3'}), 82.1, 82.1 (C₃), 79.8, 79.7 (C_{7''}), 61.9, 61.8 (C_{1''}), 60.6 (C_{8'}), 58.9, 58.8 (C_{10'}), 56.6, 56.3 (C_{7'&9'}), 46.9, 46.7 (C_{4''}), 30.9 (C_{2''}), 28.8, 28.6, 28.4 (C_{3''}), 27.3 (C_{8''&10''}), 24.5, 23.7 (C_{9''})

IR ν_{max}(ATR): 1760 cm⁻¹ (β-lactam C=O), 1692 cm⁻¹ (*N*-Boc-L-proline C=O)

HRMS: ESI calculated for C₂₉H₃₅ClN₂NaO₉ [**M+Na**⁺], 613.192329; found 613.192975, error - 1.1 ppm

1-Tert-butyl 2-((2*R*,3*R*)-2-(3-chloro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (117b)

117b was resolved from **117** using LC as described in **General Method XII**.

Yield resolved from diastereomer mixture: 112mg (0.19 mmol) 12.2%

Appearance: White amorphous powder

Melting Point: 74-77 °C

R_f: 0.24 (plate development 1), 0.37 (plate, development 2), (2:1 MTBE :*n*-hexane)

¹H NMR (600 MHz, CDCl₃): δ 7.41 (dd, 1H, *J* = 8 Hz, *J* = 1.8 Hz), 7.25 (m, 1H), 7.97 (apparent t, 1H, *J* = 8 Hz), 6.54 (s, 0.6H), 6.53 (s, 1H), 5.41 (d, 0.3H, *J* = 1.8 Hz), 5.32 (d, 0.4H, *J* = 1.8 Hz), 4.92 (apparent s, 0.5H), 4.82 (apparent s, 0.4H), 4.40 (m, 1H), 3.96 (s, 1H), 3.92 (s, 2H), 3.80 (s, 3H), 3.74 (s, 2H), 3.73 (s, 4H), 3.62-3.41 (m, 2H), 2.38 – 2.25 (m, 1H), 2.12 – 1.89 (m, 3H), 1.45 (s, 3H), 1.44 (s, 6H)

¹H NMR (600 MHz, DMSO-*d*₆): δ 7.64 (d, 1H, H_{6'}, *J* = 2.8 Hz), 7.47 (dd, 1H, H_{2'}, *J* = 8.7 Hz, *J* = 2.6 Hz, H_{2''}), 7.18 (d, 1H, *J* = 8 Hz, H_{3''}), 6.55 (s, 2H, H_{1&3}), 5.63, 5.59 (apparent ss, 1H, H₄), 5.25, 5.17, (apparent ss, 1H, H₄), 4.33 (m, 1H, H_{1''}), 3.86 (s, 3H, H_{10'}), 3.68 (s, 3H, H_{8'}), 3.33 (s, 6H, H_{7&9'}), 2.31 (m, 2H, H_{4''}), 2.02 (m, 2H, H_{2''}), 1.88 (m, 2H, H_{3''})

¹³C NMR (100 MHz; DMSO-*d*₆): δ 172.4 (C_{6''}), 172.0 (C_{5''}), 161.4 (C₂), 155.3, 155.2 (C_{4''}), 154.0 (C_{5''}), 153.2 (C_{4'&6'}), 134.9 (C_{5'}), 132.6, 13.53 (C_{2'}), 129.4, 129.3, 128.8, 128.8 (C_{1''}), 127.9, 127.8 (C_{6''}), 121.7 (C_{2''}), 113.5 (C_{3''}), 96.11, 96.01 (C_{1'&3'}), 82.0, 81.9 (C_{3'}), 79.7, 62.0, 61.7 (C₄), 60.6 (C_{8'}), 58.6, 58.5 (C_{4''}), 56.7 (C_{10'}), 56.2, (C_{7'&9'}), 46.9, 46.7, 30.0, 29.8, 28.5, 28.3, 27.3, 26.3, 24.5, 23.7

IR ν_{max}(ATR): 1757 cm⁻¹ (β-lactam C=O), 1693 cm⁻¹ (*N*-Boc-L-proline C=O)

HRMS: APCI calculated for C₂₅H₂₈ClN₂O₉ [**M+H**⁺], 535.145950; found 535.147785.

ESI calculated for C₂₉H₃₅ClN₂NaO₉ [**M+Na**⁺], 613.193189; found 613.192329, error + 1.4 ppm

1-(*Tert*-butyl) -2-(4-methoxy-3-methylphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (118**)**

118 was synthesised from **20** and *N*-Boc-L-proline by **General Method XII**.

Yield: 1.16 g (2.04 mmol) 58%

Appearance: white amorphous solid

Melting Point: 65-67 °C

R_f: 0.35 (2:1 MTBE: *n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ (diastereomers and rotamers present) 7.14 (apparent m, 2H, H), 6.83 (t, 1H, *J* = 8.1 Hz), 6.57 (s, 0.5H), 6.56 (s, 0.4H), 6.56 (s, 0.4H), 5.48 (d, 0.3H, *J* = 1.7 Hz), 5.43 (d, 0.3H, *J* = 1.5 Hz), 5.39 (d, 0.3H, *J* = 1.5 Hz), 5.36 (d, (d, 0.3H, *J* = 1.7 Hz), 4.92 (apparent s, 0.4H), 4.89 (apparent s, 0.3H), 4.82 (apparent s, 0.4H), 4.39 (m, 1H, H_{1''}), 3.84 (s, 1.2H), 3.83 (s, 0.9H), 3.82 (s, 0.7H), 3.78 (s, 0.9H), 3.78 (s, 2H), 3.72 (s, 3.5H), 3.72 (s, 2.5H), 3.6-3.4 (m, 2H), 2.3-2.0 (m, 4H), 1.46 (s, 1H, *t*-butyl), 1.43 (s, 8H, *t*-butyl, H_{8''} & H_{9''})

IR ν_{max}(ATR): 1757 cm⁻¹ (β-lactam C=O), 1693 cm⁻¹ (*N*-(Boc)-L-proline C=O)

1-(*Tert*-butyl) 2-((2*S*,3*S*)-2-(4-methoxy-3-methylphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (118a**)**

118a was resolved from **118** using LC as described in **General Method XII**.

Yield resolved from diastereomer mixture: 430 mg (0.75 mmol) 36.8 %

Appearance: white fluffy dust

Melting Point: 63 °C

R_f: 0.28 (plate development 1), 0.44 (plate development 2), 0.64 (plate development 3), (2:1 MTBE: *n*-hexane). **Appearance:** white fluffy amorphous solid

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 7.15 (d, 1H, *J* = 8.6 Hz), 7.12 (d, 1H, *J* = 8.6 Hz), 6.83 (0.5H, *J* = 8.6 Hz), 6.79 (d, 0.5H, *J* = 8.6 Hz), 6.57 (s, 2H, H_{1'&3'}), 5.43 (apparent s,

0.4H, H₃), 5.39 (apparent s, 0.5H, H₃), 4.95 (apparent s, 0.5H, H₄), 4.82 (apparent s, 0.4H, H₄), 4.38 (m, 1H, H₁...), 3.84 (s, 1.4H, H₈'), 3.82 (s, 1.7H, H₈'), 3.78 (s, 3H, H₁₀'), 3.72 (s, 6H, H₇'&9'), 3.58-3.38 (m, 2H), 2.36-1.9 (m, 4H), 1.51 (s, 6H, *t*-butyl, H₈... & H₉...), 1.47 (s, 3H, H₇...)

¹³C NMR (100 MHz, CDCl₃): δ 172.4, 171.9, 161.6, 158.8, 158.2, 153.5, 153.4, 135.08, 133.1, 128.6, 128.4, 126.6, 125.1, 125.0, 110.2, 110.1, 95.5, 95.4, 82.9, 82.8, 80.2, 80.1, 63.8, 60.9, 58.7, 58.5, 56.1, 55.4, 45.6, 46.5, 46.4, 29.9, 28.44, 24.5, 23.5

IR ν_{max}(ATR): 1756 cm⁻¹ (β-lactam C=O), 1694 cm⁻¹ (*N*-Boc-L-proline C=O)

HRMS: APCI calculated for [M+H⁺], 571.265007 found, 571.265100 error + 0.2 ppm

1-(*Tert*-butyl) 2-((2*R*,3*R*)-2-(4-methoxy-3-methylphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (118b)

118b was resolved from **118** using LC as described in **General Method XII**.

Yield resolved from diastereomer mixture: 76 mg (0.13 mmol) 6%

Appearance: white fluffy dust

Melting Point: 63 °C

R_f: 0.28 (plate development 1), 0.39 (plate development 2), 0.56 (plate development 3), (2:1 MTBE :*n*-hexane)

Appearance: white amorphous powder

¹H NMR (400 MHz, CDCl₃): δ 7.16 (apparent m, 2H), 6.83 (apparent triplet, *J* = 8.3 Hz), 6.57 (s, 1H), 6.56 (s, 1H), 5.48 (d, *J* = 1.7 Hz, 0.45H), 5.36 (d, *J* = 1.7 Hz, 0.5H), 4.89 (d, *J* = 1.5 Hz, 0.47H), 4.83 (d, *J* = 1.5 Hz, 0.47H), 3.85 (s, 1.7H), 3.84 (s, 1H), 3.79 (s, 3H), 3.73 (s, 3H), 3.73 (s, 3H), 3.73 (s, 3H), 3.65-3.4 (m, 2H), 3.17-3.1 (m, 2H), 2.37-2.26 (m, 2H), 2.19 (s, 9H, *t*-butyl, H₇..., 8'...&9')

¹³C NMR (100 MHz, CDCl₃): δ 171.7, 160.2, 153.4, 146.2, 138.3, 135.8, 134.4, 133.7, 129.4, 127.6, 125.8, 96.4, 92.5, 62.0, 56.5, 55.9, 46.9, 28.9

IR ν_{max}(ATR): 1756 cm⁻¹ (β-lactam C=O), 1694 cm⁻¹ (*N*-(Boc)-L-proline C=O)

HRMS: APCI calculated for [M + H⁺], 571.265007 found, 571.264529 error -0.8 ppm

1-(*Tert*-butyl) 2-(2-(4-(methylthio)phenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (119)

119 was synthesised from **22** and *N*-(Boc)-L-proline using **General Method XII**.

Yield: 290 mg (0.51 mmol) 32%

Appearance: white fluffy powder

R_f: 0.37 (2:1 MTBE: *n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ (diastereomers and rotamers present) 7.33 (m, 1H), 7.27 (m, 2H), 6.53 (m, 2H), 5.41 (d, 0.2H), 5.38 (d, 0.2H), 5.36 (d, 0.1H), 5.34 (d, 0.3H), 5.33 (d, 0.2H), 5.0 (apparent s 0.3H), 4.93 (apparent s, 0.3H), 4.86 (apparent s, 0.4H), 4.38 (m, 1H), 3.78 (s, 1H, OCH₃), 3.77 (s, 3H, OCH₃), 3.72 (bs, 9H, OCH₃), 3.61-3.43 (m, 2H), 2.33 – 1.85 (m, 4H), 1.42 (apparent m, 9H, H₈...-10')

1-(*Tert*-butyl) 2-((2*S*,3*S*)-2-(4-(methylthio)phenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (119a)

119a was resolved from **119** using LC as described in **General Method XII**.

Yield resolved from diastereomer mixture: 80mg (0.14 mmol) 27.4%

Appearance: white fluffy dust

R_f: 0.37 (plate development 1), 0.46 (plate development 2), 0.65 (plate development 3), (2:1 MTBE:*n*-hexane)

¹H NMR (400 MHz, DMSO-*d*₆): δ (rotamers present) 7.72 (m, 0.4H), 7.68 (m, 0.4H), 7.43 (d, 0.9H, *J* = 7.7 Hz), 7.4 Hz (d, 0.8H, *J* = 8 Hz), 7.29 (d, 1H, *J* = 8 Hz), 7.28 (d, 1H, *J* = 9Hz), 6.55 (s, 0.65H, H_{1'}&3', rotamer 1), 6.54 (s, 0.96H, H_{1'}&3', rotamer 2), 5.56 (d, 0.7H, *J* = 2.2 Hz, H₃), 5.5 (d, 0.7H, *J* = 2.2 Hz, H₃), 5.47 (d, 0.3H, *J* = 2.2 Hz, H₃), 5.20 (d, 1H, *J* = 2.2 Hz, H₄), 4.29 (m, 1H, H₄), 3.63 (s, 6H, H_{7'}&9'), 3.53 (s, 3H, H_{8'}), 3.4-3.26 (m, 2H), 2.46 (s, 3H, H_{10'}), 2.3-2.13 (m, 2H), 1.99- 1.76 (m, 2H), 3.46 (s, 2H, H_{9''}), 1.36 (s, 3H, H_{9''}), 1.35 (s, 6H, H_{8'''}&10''')

¹³C NMR (100 MHz, DMSO-*d*₆): δ 174.8, 171.4, 172.4, 172.2, 161.5, 154.1, 153.9, 153.7, 153.6, 153.2, 139.8, 139.6, 134.9, 132.6, 132.2, 132.1, 129.2, 128.2, 126.5, 126.4, 91.1, 96.0, 82.2, 82.18, 79.75, 79.74, 79.2, 79.1, 62.6, 62.5, 60.1, 59.1, 58.9, 58.8, 58.8, 56.3, 46.8, 56.7, 46.7, 28.6, 28.3

HRMS: APCI calculated for C₂₉H₃₆N₂NaO₈S [**M+Na⁺**], 595.208459 found, 595.208878, error - 0.7 ppm

APCI calculated for C₂₉H₃₆KN₂O₈S [**M+K⁺**], 611.182395, found 611.183278, error -1.4 ppm

1-(*Tert*-butyl) 2-((2*R*,3*R*)-2-(4-(methylthio)phenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (119b**)**

119b was resolved from **119** using LC as described in **General Method XII**.

Yield resolved from diastereomer mixture: 38 mg (0.06 mmol) 12%

Appearance: white fluffy dust

R_f: 0.37 (plate development 1), 0.38 (plate development 2), 0.57 (plate development 3), (2:1 MTBE:*n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 7.52-7.28 (m, 3H), 6.54 (s, 1H), 6.53 (s, 1H), 5.55 (d, *J* = 2 Hz, 0.6H), 5.51 (d, *J* = 2 Hz, 0.4H), 5.25 (d, *J* = 2 Hz 0.6H), 5.17 (d, *J* = 2 Hz 0.7H), 4.3 (m, 1H, H_{1''}), 3.63 (s, 6H, H_{7'}&9'), 3.57 (s, 3H, H_{8'}), 2.45 (s, 3H, H_{10'}), 2.69 (m, 2H), 2.33 (m, 2H), 2.03-1.69 (m, 2H), 1.38 (s, 6H, H_{8'''}&10'''), 1.34 (s, 3H, H_{9''})

¹³C NMR (100 MHz, CDCl₃): δ 172.45, 172, 161.5, 153.8, 128.2, 149.1, 138.8, 127.7, 128.3, 127.4, 126.5, 124.9, 119.5, 110.2, 96.1, 79.8, 62.8, 62.4, 60.6, 56.2, 28.6, 28.4

HRMS: APCI calculated for C₂₉H₃₆N₂NaO₈S [**M+ Na⁺**], 595.208458 found, 595.208199, error +0.3 ppm, **APCI** calculated for C₂₉H₃₆KN₂O₈S [**M+ K⁺**], 611.182395, found 611.183392, error +1.6 ppm

2-(3-Bromo-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) 1-(*tert*-butyl) (*S*)-pyrrolidine-1,2-dicarboxylate (120**)**

120 was synthesised from **21** and *N*-Boc-*L*-proline using **General Method XII**.

Yield: 810 mg (1.28 mmol) 61%

Appearance: white fluffy dust

Melting Point: 71-74 °C

R_f: 0.54 (three developments, 2:1 MTBE: *n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ (diastereomers and rotamers present) 7.59 (d, 0.4H, *J* = 1.7 Hz), 7.56 (d, *J* = 2.0 Hz, 0.6H), 7.29 (m, 1H), 6.94 (d, *J* = 8.62 Hz, 0.4H), 6.89 (d, *J* = 8.9 Hz, 0.4H), 6.54 (s, 0.65H), 6.53 (s, 0.5H), 6.51 (s, 0.6H), 5.41 (apparent s, 0.16H), 5.38 (apparent s, 0.16H), 5.36 (d, *J* = 1.7 Hz, 0.09H), 5.34 (d, *J* = 1.7 Hz, 0.3H), 5.30 (d, *J* = 1.7 Hz, 0.3H), 4.97 (apparent s, 0.3H), 4.94 (d, *J* = 1.7 Hz, 0.6H), 4.91 (apparent s, 0.25H), 4.83 (d, *J* = 1.7 Hz, 0.07H), 4.81 (apparent s, 0.35H), 4.37 (m, 1H, H_{1''}), 3.91 (s, 1H, H_{8'}), 3.9 (s, 1H, H_{8'}), 3.9 (s, 1H, H_{8'}), 3.80 (s, 2H, H_{10'}), 3.78 (s, 1H, H_{10'}), 3.73 (s, 4H, H_{7'}&9'), 3.73 (s, 2H, H_{7'}&9'), 3.48 (m, 2H), 2.34-1.88 (m, 4H), 1.52 (s, 3H, *t*-butyl), 1.46 (s, 3H, *t*-butyl), 1.44 (s, 3H, *t*-butyl)

¹³C NMR (100 MHz, CDCl₃): δ 172.4, 172.1, 161.2, 161.4, 160.9, 156.6, 156.3, 154.8, 153.6, 153.6, 135.3, 132.7, 131.5, 131.3, 128.6, 128.7, 128.3, 126.8, 126.6, 112.5, 112.4, 112.3, 112.2, 112.1, 95.5, 95.3, 95.3, 82.8, 82.7, 80.4, 80.2, 80.1, 65.8, 65.3, 62.9, 62.7, 62.5, 60.9, 59.1, 58.7, 58.55, 56.3, 56.1, 53.7, 46.7, 46.4, 42.0, 41.9, 31.0, 30.9, 30.1, 29.8, 29.1, 28.6, 28.5, 28.4, 28.3, 24.7, 24.6, 23.7, 23.6, 23.4, 23.1, 18.6, 17.3, 15.3

IR ν_{max} (ATR): 1756 cm⁻¹ (β-lactam C=O), 1691 cm⁻¹ (N-Boc-L-proline C=O)

HRMS: APCI calculated for C₂₉H₃₆BrN₂O₉ [**M**+ **H**⁺], 635.159869 found 635.158822, error + 1.6 ppm

2-((2*S*,3*S*)-2-(3-Bromo-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) 1-(*tert*-butyl) (*S*)-pyrrolidine-1,2-dicarboxylate (120a)

120a was resolved from the mixture of **120** using LC as described in **General Method XII**.

Yield resolved from diastereomer mixture: 210 mg (0.33 mmol) 26%

Appearance: white fluffy dust

Melting Point: 72-74 °C

R_f: 0.17 (plate development 1), 0.24 (plate development 2), 0.54 (plate development 3), (2:1 TBME:*n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 7.59 (d, 0.1H, *J* = 1.7 Hz, H_{6'}), (7.57 (d, 0.9H, *J* = 1.7 Hz, H_{6''}), 7.27 (dd, 1H, *J* = 9.1Hz, *J* = 2.1 Hz, H_{2''}), 6.94 (d, 0.2H, *J* = 9.1Hz, H_{3''}), 6.93 (d, 0.2H, *J* = 9.1Hz, H_{3''}), 6.89 (d, 0.6H, *J* = 9.1Hz, H_{3''}), 6.54 (s, 2H, H_{1'&3'}), 5.39 (apparent s, 0.3H, H₃), 5.37 (d, 0.12H, *J* = 1.9 Hz, H₃), 5.35 (d, 0.5H, *J* = 1.9 Hz), 4.97 (apparent s, 0.6H, H₄), 4.84 (d, 0.1H, *J* = 1.9 Hz, H₄), 4.82 (apparent s, 0.3H), 4.39 (m, 1H, H_{1''}), 3.92 (s, 1.5H, H_{8'}), 3.91 (s, 2H, H₈), 3.79 (s, 3H, H₁₀), 3.74 (s, 6H, H_{7'&9'}), 3.49 (m, 2H), 1.92 (m, 2H), 1.95 (m, 2H), 1.53 (s, 6H, *t*-butyl, H_{8''&10''}), 1.47 (s, 3H, *t*-butyl, H_{9''})

¹³C NMR (100MHz, CDCl₃): δ 172.4 (C_{6''}), 172.2 (C_{5''}), 161.1 (C₂, rotamer 1), 160.9 (C₂, rotamer 2), 156.3 (C_{4''}), 154.8 (C_{4'&6'}), 153.7, 153.6, 135.2 (C_{5'}), 132.7 (C_{2'}), 131.5 (C_{2''}), 131.3, 128.7, 128.4, 126.6 (C_{1''}), 126.5 (C_{6''}), 112.3 (C_{3''}), 112.14, 95.5 (C_{1'&3'}), 95.3, 82.7, 82.6, 82.4 (C₃), 80.2, 65.3, 62.9 (C₄), 60.95 (C_{8'}), 58.6, 56.3 (C_{10'}), 56.2 (C_{7'&9'}), 46.7, 46.4, 41.9, 29.8, 29.1, 28.5, 26.9, 24.6

IR ν_{max} (ATR): 1756 cm⁻¹ (β-lactam C=O), 1691 cm⁻¹ (N-Boc-L-proline C=O)

HRMS: APCI calculated for C₂₉H₃₆BrN₂O₉ [**M**+ **H**⁺], 635.159869 found 635.157875, error - 3.1 ppm

2-((2*R*,3*R*)-2-(3-Bromo-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) 1-(*tert*-butyl) (*S*)-pyrrolidine-1,2-dicarboxylate (120b)

120b was resolved from **120** using LC as described in **General Method XII**.

Yield recovered from diastereomer mixture: 100 mg (0.158 mmol) 12 %

Appearance: white fluffy dust

Melting Point: 73-74 °C

R_f: 0.17 (plate development 1), 0.21 (plate development 2), 0.44 (plate development 3), (2:1 TBME: *n*-hexane)

Appearance: white amorphous solid

¹H NMR (400 MHz, CDCl₃): δ 7.59 (d, 0.5H, *J* = 1.9Hz, H_{6''}), 7.57 (d, 0.5H, *J* = 1.9Hz, H_{6''}), 7.32 (dd, *J* = 8.9 Hz, *J* = 1.9Hz, 1H, H_{2''}), 6.93 (apparent triplet, 1H, *J* = 8.9 Hz, H_{3''}), 6.54 (s, 0.65H, H_{1'&3'}), 6.53 (s, 0.9H, H_{1'&3'}), 5.42 (d, *J* = 1.8 Hz, 0.43H, H₃), 5.31 (d, *J* = 1.8 Hz, 0.6H, H₃), 4.92 (apparent s, 0.6H, H₄), 4.82 (apparent s, 0.5H, H₄), 4.39 (m, 1H, H₄), 3.93 (s, 1H, H_{8'}),

3.91 (s, 2H, H_{8'}), 3.8 (s, 3H, H_{10'}), 3.75 (s, 3H, H_{7'&9'}), 3.74 (s, 3H, H_{7'&9'}), 3.58-3.41 (m, 2H), 2.37-2.19 (m, CH₂), 2.1-1.9 (m, 2H, CH₂), 1.45 (s, 3H, H_{9'''}), 1.44 (s, 6H, H_{8'''-10'''})

¹³C NMR (100 MHz, CDCl₃): δ 172.1, 161.4, 157.0, 154.4 (C_{4'&6'}), 135.5, 133.6, 131.4, 127., 113.1 (C_{3''}), 95.5 (C_{1'&3'}), 82.9 (C₃), 80.1, 62.7 (C₄), 61.5 (C₈), 58.6 (C_{1'''}), 56.7 (C_{10'}), 56.0 (C_{7'&9'}), 47.2, 39.9, 30.6, 28.8 (C_{8'''&10'''}), 28.1 (C_{9'''}), 24.0, 22.9

IR ν_{max} (ATR): 1756 cm⁻¹ (β-lactam C=O), 1691 cm⁻¹ (N-Boc-L-proline C=O)

HRMS: APCI calculated for C₂₉H₃₆BrN₂O₉ [**M**+ **H**⁺], 635.159869 found 635.157875, error - 3.1 ppm

1-(*tert*-butyl) 2-(2-(4-Fluorophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (121)

121 was synthesised from **89** and N-Boc-L-proline using **General Method XII**.

Yield: 240 mg (0.44 mmol) 44%

Appearance: pale yellow powder

R_f: 0.16 (2:1 TBME: *n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 7.38 (apparent m, 2H, H_{3''&5''}), 7.11 (m, 2H, H_{2''&6''}), 6.52 (s, 0.7H, H_{1'&3'}), 6.51 (s, 0.65H, H_{1&3'}), 6.51 (s, 0.5H, H_{1'&3'}), 5.39 (d, 0.2H, *J* = 1.5 Hz, H₃), 5.36 (d, 0.1H, *J* = 1.5 Hz, H₃), 5.35 (d, 0.06H, *J* = 1.5 Hz, H₃), 5.34 (d, 0.2H, *J* = 1.5 Hz, H₃), 5.32 (d, 0.2H, *J* = 1.5 Hz, H₃), 5.05 (d, 0.2H, *J* = 0.2H, H₄), 4.98 (0.3H, *J* = 1.4 Hz, H₄), 4.92 (d, 0.06H, *J* = 1.4 Hz, H₄), 4.89 (apparent s, 0.3H, H₄), 4.39 (m, 1H, H_{1'''}), 3.79 (s, 0.8H, H_{8'}), 3.78 (s, 2.2H, H_{8'}), 3.72 (s, 3H, H_{7'&9'}), 3.71 (s, 3H, H_{7'&9'}), 3.6- 3.3 (m, 2H), 2.43 – 1.87 (4H), 1.43 (apparent m, 9H, *t*-butyl, H_{8'''-10'''})

¹⁹F NMR (376 MHz): δ -111.6, -111.9, -112.4, -112.5, -113.6, -113.6, -113.7

1-(*tert*-butyl) 2-((2*S*,3*S*)-2-(4-Fluorophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (121a)

121a was resolved from **121** using LC as described in **General Method XII**.

Yield recovered from diastereomer mixture: 120 mg (0.22 mmol) 50%

Appearance: white fluffy amorphous dust

R_f: 0.16 (plate development 1), 0.24 (plate development 2), 0.38 (plate development 3), 0.48 (plate development 4), (2:1 MTBE:*n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ 7.36 (m, 2H), 7.13 (t, 0.8H, *J* = 9.8 Hz), 7.06 (t, 1.3H, *J* = 9.8 Hz), 6.52 (s, 1.3H, H_{1'&3'}), 6.51 (s, 1H, H_{1'&3'}), 5.37 (apparent s, 0.3H, H₃), 5.35 (d, 0.2H, *J* = 1.8 Hz H₃), 5.34 (d, 0.6H, *J* = 1.8 Hz, H₃), 5.01 (apparent s, 0.7H, H₄), 4.89 (s, 0.4H, H₄), 4.28 (m, 1H H_{1'''}), 3.79 (s, 3H, H_{8'}), 3.72 (bs, 6H, H_{7'&9'}), 3.6-3.3 (m, 2H), 2.36-1.86 (m, 4H), 1.49 (m, 9H, H_{8'''-10'''})

¹³C NMR (100 MHz, CDCl₃): δ 172.4, 164.3, 161.8, 154.6, 154.0, 134.0, 132.7, 130.8, 128.2, 116.2, 108.7, 107.6, 95.4, 82.6, 81.0, 63.3, 61.0, 58.6, 56.0, 46.8, 46.3, 30.9, 23.9

¹⁹F NMR (376 MHz, CDCl₃): δ -111.6, -111.9, -112.5, -113.6

HRMS: APCI calculated for C₂₈H₃₄FN₂O₈ [**M**+ **H**⁺], 545.229371, found 545.229371, error - 1.9 ppm

1-(*tert*-butyl) 2-((2*R*,3*R*)-2-(4-Fluorophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (S)-pyrrolidine-1,2-dicarboxylate (121b)

121b was resolved from **121** using LC as described in **General Method XII**.

Yield recovered from diastereomer mixture: 80 mg (0.15mmol) 34%

Appearance: white fluffy amorphous dust

R_f: 0.16 (plate development 1), 0.24 (plate development 2), 0.33 (plate development 3), 0.43 (plate development 4), (2:1 MTBE : *n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ 7.40 (m, 2H), 7.09 (m, 2H), 6.52 (s, 0.3H, H_{1'&3'}), 6.52 (s, 0.3H, H_{1'&3'}), 6.51 6.52 (s, 1.5H, H_{1'&3'}), 5.34 (apparent s, 0.4H, H₃), 5.31 (d, 0.6H, *J* = 1.3 Hz, H₃), 4.98 (apparent s, 0.4H, H₄), 4.89 (apparent s, 0.4H, H₄), 4.39 (m, 1H, H_{1''}), 3.79 (s, 3H, H_{8'}), 3.71 (bs, 6H, H_{7'&9'}), 3.6-3.4 (m, 2H), 2.39-1.88 (m, 4H), 1.5 (s, 3H, *t*-butyl, H_{9'''}), 1.43 (s, 6H, H_{8'''&10'''})

¹³C NMR (100 MHz, CDCl₃): δ 172.1, 161.2, 154.7, 153.6, 135.2, 132.7, 128.5, 128.2, 126.4, 117.1, 116.5, 116.2, 114.9, 111.3, 107.6, 95.3, 82.7, 80.3, 63.1, 61.0, 58.9, 56.0, 56.0, 46.5, 30.9, 29.9, 28.3, 23.7, 20.7

¹⁹F NMR (376 MHz): δ -111.6, -112.4, -113.4, -113.5

HRMS: APCI calculated for C₂₈H₃₄FN₂O₈ [**M+H**⁺], 545.229371 found 545.230533, error - 2.1 ppm

1-(*tert*-butyl) 2-(2-(4-Methoxy-3-nitrophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (122**)**

122 was synthesised from **63** using **General Method XII**.

Yield: 360mg (0.6 mmol) 60%

Appearance: white fluffy amorphous solid

R_f: 0.15 (2:1 TBME:*n*-hexane), white fluffy amorphous solid

¹H NMR (400 MHz, CDCl₃): δ (mixed diastereomers and rotamers present) 7.92 (d, 0.1H, *J* = 2.9 Hz, H_{6''}, rotamer 1), 7.90 (d, 0.7H, *J* = 2.9 Hz, H_{6''}), 7.88 (d, 0.2H, *J* = 2.9 Hz, H_{6''}), 7.61 (dd, 0.4H, *J* = 7.9 Hz, 2.1 Hz, H_{3''}), 7.55 (dt, 0.6H, *J* = 7.9 Hz, 2.1 Hz, H_{3''}), 7.18 – 7.13 (apparent m, 0.7H, H_{2''}), 7.11 (d, 0.3H, *J* = 8.9 Hz, H_{2''}), 6.54 (s, 0.4H, H_{1'&3'}), 6.53 (s, 0.25H, H_{1'&3'}), 6.52 (s, 0.3H, H_{1'&3'}), 6.51 (s, 1H, H_{1'&3'}), 5.36 (apparent bs, 0.2H, H₃), 5.35 (apparent bs, 0.1H, H₃), 5.32 (apparent m, 0.3H, H₃), 5.29 (d, 0.35H, *J* = 1.7 Hz, H₃), 5.07 (d, 0.2H, H₄), 5.01 (d, 0.3H, H₄), 4.92 (d, 0.1H, H₄), 4.88 (apparent bs, 0.3H, H₄), 4.39 (m, 1H, H₄), 4.01 (s, 0.8H, OCH₃, H_{8'}), 4.00 (s, 0.8H, OCH₃, H_{8'}), 3.99 (s, 0.9H, OCH₃, H_{8'}), 3.98 (s, 0.6H, OCH₃, H_{8'}), 3.81 (s, 1H, OCH₃, H_{10'}), 3.80 (s, 2H, OCH₃, H_{10'}), 3.76 (s, 2H, H_{1'&3'}), 3.75 (s, 2H, H_{1'&3'}), 3.75 (s, 2H, H_{1'&3'}), 3.62-3.40 (m, 2H), 2.37-1.91 (m, 4H), 1.53-1.44 (m, 9H, H_{8'''-10'''})

¹³C NMR (100 MHz, CDCl₃): δ 172.2, 160.8, 154.6, 153.8, 153.7, 153.5, 153.2, 139.9, 132.45, 132.5, 132.4, 131.8, 127.7, 127.3, 124.1, 123.9, 114.6, 114.4, 114.2, 95.6, 95.4, 82.7, 82.5, 80.5, 80.4, 77.3, 72.8, 62.7, 62.1, 59.2, 58.7, 58.6, 56.2, 56.2, 49.5, 49.7, 46.4, 38.7, 31.1, 29.9, 28.4, 28.3, 27.0, 24.8, 24.7

HRMS: APCI calculated for C₂₉H₃₄N₃O₁₁ [**M-H**⁺], 600.219882, found 600.219239, error -1.1 ppm

1-(*tert*-butyl) 2-((2*S*,3*S*)-2-(4-Methoxy-3-nitrophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (122a**)**

122a was resolved from **122** using LC as described in **General Method XII**.

Yield resolved from diastereomer mixture: 160 mg (0.27 mmol) 27%

R_f: 0.15 (plate development 1), 0.33 (plate development 2), 0.6 (plate development 3), (2:1 TBME:*n*-hexane), white fluffy amorphous solid

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 7.88 (d, 1H, *J* = 2.9 Hz, H_{6''}), 7.54 (d, 1H, *J* = 9.9 Hz, 2.2 Hz, H_{2''}), 7.17 (d, 0.3H, *J* = 10.8 Hz, H_{3''}), 7.12 (d, 0.7H, *J* = 8.1 Hz, H_{3''}), 6.54 (s, 1.3H, H_{1'&3'}), 6.52 (s, 0.8H, H_{1'&3'}), 5.36 (apparent s, 0.3H, H₃), 5.3 (s, 0.8H, H₃), 5.07 (s, 0.7H, H₄), 4.89 (s, 0.3H, H₄), 4.38 (m, 1H, H_{1''}), 4.0 (s, 1H, H_{10'}), 3.98 (s, 2H, H_{10'}), 3.81 (s, 3H, H_{8'}),

3.76 (s, 6H, H_{7'&9'}), 3.59-3.42 (m, 2H), 2.4-1.89 (m, 4H), 1.52 (s, 6H, *t*-butyl, H_{8'''&10'''}), 1.48 (s, 3H, H_{9'''})

¹³C NMR (100 MHz, CDCl₃): δ 172.8, 172.3, 160.7, 160.4, 154.6, 153.8, 153.7, 153.4, 153.2, 139.8, 132.3, 131.9, 131.7, 127.7, 127.3, 124.1, 123.8, 114.5, 114.1, 95.6, 95.2, 82.7, 82.6, 80.3, 65.8, 62.6, 61.0, 58.6, 56.8, 56.7, 56.4, 46.7, 46.4, 38.7, 31.0, 29.9, 24.6, 23.5, 18.7, 17.6, 15.3

HRMS: APCI calculated for C₂₉H₃₄N₃O₁₁ [**M-H**⁺], 600.219882, found 600.219239, error -1.1 ppm

1-(*tert*-butyl) 2-((2*R*,3*R*)-2-(4-Methoxy-3-nitrophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) (*S*)-pyrrolidine-1,2-dicarboxylate (122b**)**

122b was resolved from **122** using LC as described in using **General Method XII**.

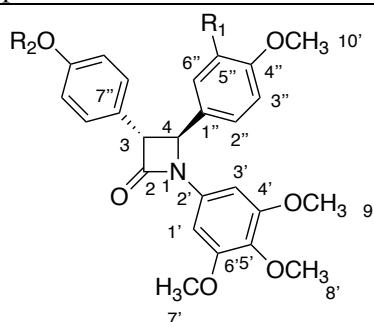
Yield: 100mg (0.17 mmol) 17%

R_f: 0.15 (plate development 1), 0.29 (plate development 2), 0.48 (plate development 3), (2:1 TBME:*n*-hexane), white fluffy amorphous solid

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 7.92 (d, 0.1H, *J* = 1.9 Hz, H_{6''}), 7.90 (d, 0.8H, *J* = 1.9 Hz, H_{6''}), 7.6 (dd, 0.6H, *J* = 9.9 Hz, 2.2 Hz, H_{2''}), 7.5 (dd, 0.6H, *J* = 9.9 Hz, 2.2 Hz, H_{2''}), 7.2 (apparent m, 1H, H_{3''}), 6.52 (s, 0.3H, H_{1'&3'}), 6.52 (s, 0.6H, H_{1'&3'}), 6.51 (s, 0.9H, H_{1'&3'}) 5.37 (d, 0.3H *J* = 1.5 Hz, H₃), 5.32 (d, 0.15H, *J* = 2.2 Hz, H₃), 5.29 (d, 0.4H, *J* = 2.2 Hz, H₃), 5.01 (d, 0.5H, *J* = 2.2 Hz, H₄), 4.92 (d, 0.1H, *J* = 2.2 Hz, H₄), 4.88 (apparent s, 0.3H, H₄), 4.39 (m, 1Hm H_{1'''}), 4.05 (s, 0.5H, H_{10'}), 4.01 (s, 0.5H, H_{10'}), 3.99 (s, 1.5H, H_{10'}), 3.80 (s, 3H, H_{8'}), 3.76 (s, 3H, H_{7'&9'}), 3.75 (s, 3H, H_{7'&9'}), 3.67-3.42 (m, 2H), 2.38-1.91 (m, 4H), 1.44 (s, 3H, *t*-butyl, H_{9'''}), 1.48 (s, 6H, H_{8'''&10'''})

¹³C NMR (100 MHz, CDCl₃): δ 172.4, 172.2, 169.9, 160.9, 160.9, 154.7, 153.8, 153.5, 153.4, 153.2, 139.8, 135.6, 135.4, 132.5, 132.3, 132.1, 131.8, 131.7, 127.7, 127.5, 127.3, 124.1, 123.9, 123.8, 114.6, 114.4, 114.3, 95.4, 95.3, 82.7, 82.6, 80.5, 80.3, 62.7, 62.5, 62.1, 61.09, 59.2, 58.7, 56.8, 56.7, 56.2, 56.1, 46.7, 46.4, 31.1, 29.8, 28.4, 28.3, 27.0, 24.8, 23.8, 20.4

Numbering nomenclature for 3-phenolic diastereomers



Code	R ₁	R ₂
134	F	<i>N</i> -Boc-L-Proline
135	H	<i>N</i> -Boc-L-Proline
136	F	<i>N</i> -Boc-L-Phenylalanine

1-(*tert*-butyl) 2-(4-(2-(3-Fluoro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl)phenyl) (*2S*)-pyrrolidine-1,2-dicarboxylate hydrate (134**)**

134 was synthesised from **68** and *N*-Boc-L-Proline using **General Method XII**.

Yield: 80%

R_f: 0.25 (2:1 *n*-hexane: ethyl acetate), 0.83 (2:1 ethyl acetate: *n*-hexane)

¹H NMR (400 MHz, DMSO-*d*₆): δ 7.45 -7.32 (apparent m, 3H), 7.17 (d, 2H, *J* = 8 Hz), 6.95 (d, 1H, *J* = 7.9 Hz), 6.62 (s, 2H, H_{1'&3'}), 5.29 (d, 0.6 H, *J* = 1.7 Hz), 5.27 (d, 0.4H, *J* = 1.7 Hz), 4.55 (d, 1H, *J* = 1.7 Hz), 4.14 (m, 1H), 3.84 (s, 3H), 3.67 (s, 6H, H_{7'&9'}), 3.59 (s, 3H), 3.4-3.2 (m, 2H), 2.15 -1.83 (m, 4H), 1.43 (s, 3H), 1.39 (s, 6H, *t*-butyl)

¹⁹F NMR (376 MHz, DMSO-*d*₆): -134.33

1-(*tert*-butyl) 2-(4-(2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl)phenyl) (2*S*)-pyrrolidine-1,2-dicarboxylate hydrate (135)

135 was synthesised from **69** and *N*-Boc-L-proline using **General Method XII**.

Yield: 71 %

R_f: 0.56 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, DMSO-*d*₆): δ 7.49 (d, 2H, *J* = 8.3 Hz), 7.45 (d, 1H, *J* = 7.8 Hz), 7.16 (apparent doublet, 1H, *J* = 8.3 Hz), 7.16 (d, 1H, *J* = 1.8 Hz, H_{6'}), 6.99 (d, 2H, *J* = 8.3 Hz), 6.6 (s, 2H, H_{7'&9'}), 5.27 (d, 0.6H, *J* = 1.7 Hz), 5.25 (apparent s, 0.4H), 4.5 (apparent s, 1H), 4.42 (m, 1H), 3.76 (s, 3H), 3.65 (s, 6H), 3.58 (s, 3H), 2.41-2.2 (m, 2H), 2.16-1.86 (m, 4H), 1.42 (s, 3H, *t*-butyl), 1.34 (s, 6H, *t*-butyl)

4-(2-(3-fluoro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl)phenyl(*tert*-butoxycarbonyl)-L-phenylalaninate (136)

136 was synthesised from **68** and *N*-Boc-L-phenylalanine using **General Method XII**.

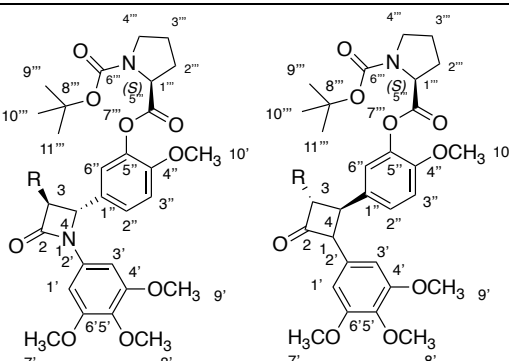
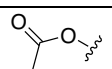
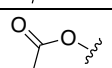
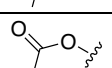
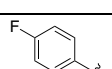
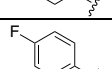
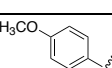
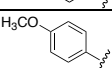
Yield: 250 mg (0.37 mmols) 81.6%

Appearance: yellow oil

R_f: 0.66-0.71 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, DMSO-*d*₆): δ 7.58-7.13 (complex m, 11H, Ar-H), 7.03 (dd, 1H, *J* = 2.2 Hz, 8.6 Hz, H_{2'}), 6.6 (s, 2H, H_{1'&3'}), 5.28 (apparent s, 1H, H₃), 4.53 (apparent s, 1H, H₄), 4.38 (m, phenylalanine CH), 3.84 (s, 3H, H_{10'}), 3.67 (s, 6H, H_{7'&9'}), 3.59 (s, 3H, H_{8'}), 3.07 (apparent m), 1.37 (s, 9H, *t*-butyl)

Numbering nomenclature for B ring *meta* hydroxyl proline esterified diastereomers

			
Code	3X	4X	R
123	Diastereomer mixture		
123a	<i>S</i>	<i>S</i>	
123b	<i>R</i>	<i>S</i>	
124	Diastereomer mixture		
124a	<i>S</i>	<i>R</i>	
124b	<i>R</i>	<i>S</i>	
125	Diastereomer mixture		H
125a	Nd		H
125b			H
126	Diastereomer mixture		F
127	Diastereomer mixture		
127a	<i>S</i>	<i>R</i>	
127b	<i>R</i>	<i>S</i>	
128	Diastereomer mixture		
128a	<i>S</i>	<i>R</i>	
128b	<i>R</i>	<i>S</i>	
129	Diastereomer mixture		
130	Diastereomer mixture		
130a	<i>S</i>	<i>R</i>	
130b	<i>R</i>	<i>S</i>	
131	Diastereomer mixture		
131a	<i>S</i>	<i>R</i>	
131b	<i>R</i>	<i>S</i>	

2-(5-(3-Acetoxy-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)-2-methoxyphenyl) 1-(tert-butyl) (2S)-pyrrolidine-1,2-dicarboxylate (123)

123 was synthesised from **67b** and *N*-Boc-L-proline using **General Method XII**.

Yield: 380mg (0.62 mmol) 65%

Appearance: white fluffy dust

R_f: 0.2 (2:1 TBME: *n*-hexane)

¹H NMR (400 MHz, DMSO-*d*₆): δ (diastereomers and rotamers present) 7.41 (m, 1H), 7.28 (d, *J*_{6''-2''} = 2.2 Hz, 0.15H, H_{6''}), 7.24 (d, *J*_{6''-2''} = 2.2 Hz, 0.2H, H_{6''}), 7.19 (dd, 1H, *J*_{2''-6''} = 2.2 Hz, *J*_{2''-3''} = 7.6 Hz, H_{2''}), 7.16 (2Xd, 1H, *J*_{3''-2''} = 7.6 Hz, H_{3''}), 6.53 (s, 1H, H_{1'&3'}), 6.52 (s, 1H, H_{1'&3'}), 5.51 (d, *J*₃₋₄ = 2 Hz, 0.05H, H₃), 5.48 (d, *J*₃₋₄ = 2 Hz, 0.2H, H₃), 5.45 (m, *J*₃₋₄ = 0.5H, H₃), 5.29 (d, *J*₄₋₃ = 2 Hz, 0.3H, H₄), 5.26 (d, *J*₄₋₃ = 2 Hz 0.35H, H₄), 5.24 (m, 0.35H, H_{1''}), 4.43 (m, 1H, H_{8'}), 3.77 (s, 2H, H_{10'}), 3.76 (s, 1H, H_{10'}), 3.64 (s, 2H, H_{7'&9'}), 3.62 (s, 2H, H_{7'&9'}), 3.61 (s, 2H, H_{7'&9'}), 3.58 (s, 3H, H_{8'}), 4.49 – 3.8 (m, 2H), 2.38 – 1.7 (m, 4H), 1.4 (s, 1.5H), 1.39 (s, 1.5H), 1.34 (s, 6H, *t*-butyl), 1.29 (s, 3H, *t*-butyl)

¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.1, 170.9, 165.3 (C₂), 161.9, 161.8, 154.0, 153.9, 153.3, 151.6, 151.5, 139.6, 139.6, 139.5, 134.8, 132.2, 131.9, 130.1, 128.9, 128.5, 128.5, 128.4, 126.5, 126.3, 126.2, 125.5, 122.4, 122.2, 121.8, 121.5, 120.3, 115.9, 115.6, 113.8, 113.7, 95.9, 95.89, 95.84, 82.2, 82.1, 81.9, 81.8, 79.6, 79.5, 66.1, 62.1, 62.0, 60.5, 58.9, 58.8, 56.6, 56.4, 56.2, 46.9, 46.7, 38.7, 31.0, 28.5, 28.3, 28.2, 24.4, 23.6, 20.7

HRMS: APCI calculated for C₃₁H₃₇N₂O₁₁ [**M** + **H**⁺], 613.240284; found 613.23886, error + 2.0 ppm

2-(5-((2S,3S)-3-Acetoxy-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)-2-methoxyphenyl) 1-(tert-butyl) (S)-pyrrolidine-1,2-dicarboxylate (123a)

123a was resolved from **123** using LC as described in **General Method XII**.

Yield resolved from diastereomer mixture: 100mg (0.16 mmol) 26%

Appearance: white fluffy dust

R_f: 0.2 (plate development 1), 0.45 (plate development 2) (2:1 TBME: *n*-hexane)

¹H NMR (600 MHz, DMSO-*d*₆): δ 7.41 (apparent triplet, *J* = 2.3 Hz, 0.5H, H_{6''}), 7.39 (apparent triplet, *J* = 2.3 Hz, 0.5H, H_{6''}, rotamer 2), 7.39 (d, 0.3H, *J* = 2.8 Hz, H_{6''}), 7.25 (apparent triplet, 0.5H, *J* = 2.3 Hz), 7.19 (m, 1H), 7.16 (d, 0.7H, *J* = 8.1 Hz, H_{3''}), 6.53 (s, 1.4H, H_{1'&3'}), 6.52 (s, 0.6H, H_{1'&3'}), 5.51 (d, *J* = 1.6 Hz, 0.3H, H₃), 5.48 (apparent m, 0.2H), 5.45 (d, *J* = 1.6 Hz, 0.5H, H₃), 5.29 (d, *J* = 1.6 Hz, 0.4H, H₄), 5.27 (d, *J* = 1.6 Hz, H₄), 5.25 (d, *J* = 1.6 Hz, 0.4H, H₄), 3.77 (s, 2H, H_{10'}), 3.76 (s, 1H, H_{10'}), 3.63 (s, 6H, H_{7'&9'}), 3.56 (s, 3H, H_{8'}), 2.17 (s, OCH₃)

¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.1 (C_{8''}), 170.9 (C_{5''}), 169.9, 161.9 (C₂), 154.6 (C_{4'&6'}), 151.6 (C_{4''}), 139.5 (C_{5'}), 134.8 (C_{5'}), 132.8 (C_{2'}), 128.4 (C_{1''}), 126.3 (C_{2''}), 121.8 (C_{6''}), 113.8 (C_{3''}), 95.8 (C_{1'&3'}), 82.0 (C₃), 81.8 (C₃), 79.6, 79.3, 62.0 (C₄), 60.6 (C_{8'}), 58.9, 56.7 (C_{7'&9'}), 56.4 (C_{10'}), 56.2 (C_{10'}), 46.9, 46.6, 30.9, 28.6, 28.2

HRMS: APCI calculated for C₃₁H₃₇N₂O₁₁ [**M** + **H**⁺], 613.240284; found 613.23886, error + 2.3 ppm

2-(5-((2R,3R)-3-Acetoxy-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)-2-methoxyphenyl) 1-(tert-butyl) (S)-pyrrolidine-1,2-dicarboxylate (123b)

123b was resolved from **123** using LC as described in **General Method XII**.

Yield resolved from diastereomer mixture: 80mg (0.13 mmol) 21%

Appearance: white fluffy dust

R_f: 0.2 (plate development 1), 0.39 (plate development 2) (2:1 TBME: *n*-hexane)

¹H NMR (600 MHz, DMSO-*d*₆): δ (rotamers present) 7.41 (d, 0.25H, *J* = 2.4 Hz, H_{6''}), 7.40 (d, 0.3H, *J* = 2.4 Hz, H_{6''}), 7.38 (d, 0.15H, *J* = 2.4 Hz, H_{6''}), 7.28 (d, 0.1H, *J* = 2.1 Hz, H_{6''}), 7.25 (d, 0.25H, *J* = 2.1 Hz, H_{6''}), 7.19 (d, *J* = 8 Hz, 1H, H_{3''}), 7.16 (dd, 1H, *J* = 7.3 Hz, *J* = 2.1 Hz, H_{3''}), 6.53 (s, 0.8H, H_{1'&3'}), 6.52 (s, 1.2H, H_{1'&3'}), 5.51 (d, 0.1H *J* = 1.8Hz, H₃), 5.48 (d, 0.3H, *J* = 1.8Hz, H₃), 5.49 (d, 0.6H, *J* = 1.8 Hz, H₃), 5.29 (d, 0.2H *J* = 1.8Hz, H₄), 5.26 (d, 0.5H, *J* = 1.8Hz, H₄), 5.25 (d, 0.3H, *J* = 1.8Hz, H₄), 4.42 (m, 1H, H_{1'''}), 3.78 (s, 2H, H_{10'}), 3.77 (s, 1H, H_{10'}), 3.64 (s, 2H, H_{7'&9'}), 3.63 (s, 1H, H_{7'&9'}), 3.62 (s, 3H, H_{8'}), 3.42-3.33 (m, 2H), 2.39-2.26 (m, 2H), 1.94-1.85 (m, 2H), 1.39 (s, 3H, H_{10'''}), 1.29 (s, 6H, H_{9'''&11'''})

¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.1, 170.9, 169.9, 165.1, 161.9, 154.0, 153.6, 153.3, 151.6, 161.5, 139.6, 139.5, 134.8, 133.9, 132.7, 128.5, 126.5, 126.2, 122.3, 121.5, 113.8, 95.9, 95.8, 95.2, 82.1, 81.9, 79.57, 62.1, 62.0, 59.0, 56.5, 56.2, 55.9, 46.9, 46.7, 40.5, 40.4, 39.9, 28.5, 28.3, 28.2, 20.7

HRMS: APCI calculated for C₃₁H₃₇N₂O₁₁ [**M** + **H**⁺], 613.240284; found 613.239433 error + 1.4 ppm

1-(*Tert*-butyl) 2-(2-methoxy-5-(4-oxo-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (*S*)-pyrrolidine-1,2-dicarboxylate (124**)**

124 was synthesised from **24** and *N*-Boc-L-proline using **General Method XII**.

Yield: 363 mg (0.57 mmol) 57%

Melting Point: 77-82 °C

¹H NMR (400 MHz, CDCl₃): δ (diastereomers and rotamers present) 7.44 – 7.33 (m, 5H), 7.30 – 7.25 (m, 2H), 7.23 (d, 0.3H, *J* = 2.1Hz, H_{6''}), 7.19 (d, 0.3H, *J* = 2.1Hz, H_{6''}), 7.08 (apparent triplet, 0.6H), 7.01 (apparent triplet, 1H), 6.61 (s, 2H, H_{1'&3'}), 4.86 (m, 1H, H₃), 4.59 (m, 0.4H, H_{1'''}), 4.48 (m, 0.6H, H_{1'''}), 4.34 (d, 0.2H, *J* = 1.9 Hz, H₄), 4.31 (d, 0.2H, *J* = 1.9 Hz, H₄), 4.29 (apparent s, 0.6H, H₄), 3.86 (s, 1.6H, H_{8'}), 3.85 (s, 1.1H, H_{8'}), 3.84 (s, 0.5H, H_{8'}), 3.79 (s, 3H, H₁₀), 3.73 (s, 6H, H_{7'&9'}), 3.66- 3.4 (m, 2H), 2.4-1.9 (m, 4H), 1.5-1.39 (5 X s, 9H, H_{8'''&11'''})

¹³C NMR (100 MHz, CDCl₃): δ 170.9, 170.8, 169.1, 165.5, 165.4, 153.7, 153.6, 153.5, 151.4, 140.2, 134.6, 133.6, 130.1, 129.5, 128.0, 127.9, 127.4, 124.4, 124.1, 121.5, 121.3, 120.4, 120.2, 113.1, 113.0, 97.6, 94.8, 80.1, 79.9, 72.8, 65.1, 64.9, 63.44, 61.0, 59.0, 58.99, 56.0, 55.9, 49.5, 46.7, 46.4, 31.1, 30.9, 30.0, 28.4, 28.3, 27.0, 24.4, 23.6

IR ν_{max}(ATR): 1751 cm⁻¹ (β-lactam C=O), 1693 cm⁻¹ (*N*-Boc-L-proline C=O)

1-(*Tert*-butyl) 2-(2-methoxy-5-((2*R*,3*S*)-4-oxo-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (*S*)-pyrrolidine-1,2-dicarboxylate (124a**)**

124a was resolved from **124** using LC as described in **General Method XII**.

Yield: 93 mg (0.15 mmol) 26%

Appearance: white fluffy dust

Melting Point: 73-77 °C

¹H NMR (600 MHz, DMSO-*d*₆): δ 7.38 (m, 6H), 7.21 (d, 2H, *J* = 12 Hz), 6.61 (s, 2H, H_{1'&3'}), 5.30, 4.95, (1H, apparent s, H₃), 4.52, 4.45 (1H, apparent s, H₄), 4.41 (m, H_{1'''}), 3.78, 3.77 (s, 3H, H_{8'}), 3.64 (s, 6H, H_{7'&9'}), 3.59 (s, 3H, H_{10'}), 1.40 (s, 3H, H_{10'''}), 1.34 (s, 6H, H_{8'''&11'''})

¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.2, 170.8, 165.8, 165.7, 165.7, 153.9, 153.6, 153.3, 151.4, 148.5, 147.4, 139.7, 139.7, 139.6, 135.2, 134.4, 133.4, 130.4, 130.3, 129.9, 128.3, 128.2, 128.0, 125.9, 122.0, 121.3, 114.1, 114.0, 95.5, 93.9, 79.6, 79.5, 64.1, 63.9, 61.8, 61.7, 60.5, 59.0, 58.9, 56.5, 56.4, 56.1, 46.9, 46.7, 31.3, 30.0, 28.5, 28.4, 28.3, 27.3, 24.4, 23.6

HRMS: APCI calculated for C₃₅H₄₀N₂O₉ [**M** + **Na**⁺], 655.26202; found 655.262713, error -0.2 ppm

IR $\nu_{\max}$ (ATR): 1750 cm^{-1} (β -lactam C=O), 1694 cm^{-1} (*N*-Boc-L-proline C=O)

1-(*Tert*-butyl) 2-(2-methoxy-5-((2*S*,3*R*)-4-oxo-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (*S*)-pyrrolidine-1,2-dicarboxylate (124b**)**

124b was resolved from **124** using LC as described in **General Method XII**.

Yield: 74 mg (0.12 mmol) 21 %

Appearance: white fluffy dust

Melting Point: 66-71 $^{\circ}\text{C}$

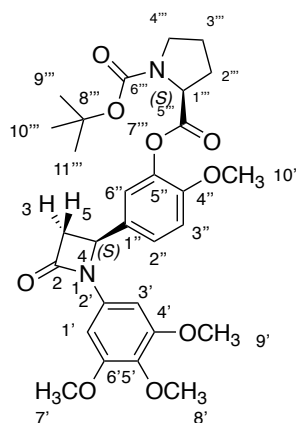
^1H NMR (600 MHz, DMSO- d_6): δ 7.46-7.40 (m, 3H), 7.37-7.32 (m, 4H), 7.21 (m, 2H), 7.20 (m, 2H), 6.60 (2 X s, 2H, $\text{H}_{1' \& 3'}$), 5.30 (broad apparent s, 1H, H_3), 4.48 (apparent s, 0.3H, H_4), 4.46 (apparent s, 0.6H, H_4), 4.42 (m, $\text{H}_{1''}$), 3.78 (s, 1.5H, H_8), 3.77 (s, 1.5H, H_8), 3.65 (s, 6H, $\text{H}_{7' \& 9'}$), 3.64 (s, 3H, $\text{H}_{7' \& 9'}$), 3.58 (s, 3H, $\text{H}_{10'}$), 1.42 (2H), 1.38 (3H), 1.33 (1H), 1.29 (3H)

^{13}C NMR (100 MHz, DMSO- d_6): δ 171.2, 170.9, 169.4, 165.7, 165.6, 154.0, 153.6, 153.3, 153.2, 151.4, 139.8, 139.7, 136.4, 135.8, 134.4, 133.4, 130.4, 129.6, 129.4, 128.8, 128.0, 127.0, 126.1, 125.9, 121.7, 121.3, 121.0, 114.1, 113.9, 97.2, 95.5, 95.5, 79.6, 79.5, 64.1, 63.9, 61.8, 61.7, 60.6, 60.5, 59.0, 58.9, 56.5, 56.2, 56.1, 49.2, 46.9, 43.9, 31.4, 30.9, 29.9, 28.5, 28.3, 27.3, 24.4, 23.6, 23.6, 22.5

IR $\nu_{\max}$ (ATR): 1752 cm^{-1} (β -lactam C=O), 1692 cm^{-1} (*N*-Boc-L-proline C=O)

HRMS: APCI calculated for $\text{C}_{35}\text{H}_{40}\text{N}_2\text{O}_9$ [$\text{M} + \text{Na}^+$], 655.26202; found 655.262619, error - 0.0 ppm

1-(*Tert*-butyl) 2-(2-methoxy-5-(4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (*2S*)-pyrrolidine-1,2-dicarboxylate (125**)**



125 was synthesised from **27** and *N*-Boc- L-proline using **General Method XII**.

Yield: 724 mg (1.27 mmol) 53%

Appearance: white/yellow fluffy dust

Melting Point: 75 $^{\circ}\text{C}$

R_f: 0.26 (3:1 TBME:*n*-hexane)

^1H NMR (600 MHz, CDCl_3): δ 7.26 (dd, 0.7H, $J_{2''-3''}=2.5$ Hz, $J_{2''-6''}=9.7$ Hz, $\text{H}_{2''}$), 7.22 (dd, 0.35H, $J_{2''-3''}=2.5$ Hz, $J_{2''-6''}=9.7$ Hz, $\text{H}_{2''}$), 7.20 (d, 0.25H, $J_{6''-2''}=2.5$ Hz, $\text{H}_{6''}$), 7.18 (d, 0.25H, $J_{6''-2''}=2.5$ Hz, $\text{H}_{6''}$), 7.05 (d, 0.5H, $J_{6''-2''}=2.5$ Hz, $\text{H}_{6''}$), 6.99 (d, 0.5H, $J_{6''-2''}=2.5$ Hz, $\text{H}_{6''}$), 6.97 (d, 1H, $J_{3''-2''}=9.7$ Hz, $\text{H}_{3''}$), 6.54 (d, 2H, $\text{H}_{1' \& 3'}$), 4.93 (m, 0.5H, H_4), 4.90 (m, 0.5H, H_4), 4.58 (m, 0.5H, $\text{H}_{1''}$), 4.48 (m, 0.5H, $\text{H}_{1''}$), 3.88 (s, 2H), 3.84 (s, 1H), 3.78 (s, 3H), 3.74 (s, 1H), 3.74 (s, 5H), 3.52 (m, 4H, proline CH_2 & H_5), 3.0 (dd, 0.5H, $J_{3-4}=2.9$ Hz, $J_{3-5}=14$ Hz, H_3), 2.9 (dd, 0.5H,

$J_{3-4} = 2.9$ Hz, $J_{3-5} = 14$ Hz, H), 2.41-2.26 (m, 2H), 2.12-1.97 (m, 2H), 1.50 (s, 3H, *t*-butyl), 1.48 (s, 3H, *t*-butyl), 1.45 (s, 3H, *t*-butyl), 1.41 (s, 3H, *t*-butyl)

^{13}C NMR (100 MHz, CDCl_3): δ 170.9, 170.8, 170.7, 164.4 ($\text{C}_{2''}$), 164.3 ($\text{C}_{2''}$), 154.4, 153.8, 153.6, 153.5, 151.3, 151.3, 140.2, 140.1, 134.5, 134.4, 134.3, 134.0, 133.9, 133.7, 130.7, 130.7, 130.7, 124.4, 123.9, 123.9, 121.7, 121.6, 121.5, 120.5, 120.3, 113.1, 113.0, 94.5, 94.4, 80.1, 79.9, 79.9, 59.0, 59.0, 58.9, 58.8, 56.0, 55.9, 53.8, 47.0, 46.8, 46.6, 46.5, 38.6, 31.0, 29.9, 28.3

IR ν_{max} (ATR): 1749 cm^{-1} (β -lactam C=O), 1694 cm^{-1} (*N*-Boc-L-Proline C=O)

1-(*Tert*-butyl) 2-(2-methoxy-5-((*S*)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (*S*)-pyrrolidine-1,2-dicarboxylate (125a)

125a was resolved from **125** LC described in **General Method XII**.

Yield resolved from diastereomer mixture: 106 mg (0.19 mmol) 15 %

Appearance: white fluffy amorphous dust

Melting Point: 75 °C

R_f: 0.26 (plate development 1), 0.44 (plate development 2), 0.56 (plate development 3), (4:1 TBME:*n*-hexane)

^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ (rotamers present) 7.40 (dd, 0.4H, $J_{2''-6''} = 2.5$ Hz, $J_{2''-3''} = 9.7$ Hz, $\text{H}_{2''}$), 7.38 (dd, 0.6H, $J_{2''-6''} = 2.5$ Hz, $J_{2''-3''} = 9.7$ Hz, $\text{H}_{2''}$), 7.20 (d, 0.3H, $J_{6''-2''} = 2.5$ Hz, $\text{H}_{6''}$), 7.21 (d, 0.1H, $J_{6''-2''} = 2.5$ Hz, $\text{H}_{6''}$), 7.18 (d, 0.3H, $J_{3''-2''} = 9.7$ Hz, $\text{H}_{3''}$), 7.16 (d, 0.2H, $J_{3''-2''} = 9.7$ Hz, $\text{H}_{3''}$), 7.14 (d, 0.3H, $J_{3''-2''} = 9.7$ Hz, $\text{H}_{3''}$), 7.13 (d, 0.22H, $J_{3''-2''} = 9.7$ Hz, $\text{H}_{3''}$), 6.53 (s, 0.45H, $\text{H}_{1' \& 3'}$), 6.52 (s, 1.1H, $\text{H}_{1' \& 3'}$), 5.19 (m, 0.4H, $\text{H}_{1''}$), 4.42 (dd, 1H, $^2J_{5-3} = 9$ Hz, $^3J_{5-4} = 4$ Hz, H_5), 3.77 (s, 2H), 3.75 (s, 1H), 3.63 (s, 3H), 3.57 (s, 3H), 3.40 (m, 1H, H_4), 2.97 (dd, 0.4H, $J_{3-4} = 2.9$ Hz, $J_{3-5} = 14$ Hz, H_3), 2.91 (dd, 0.6H, $J_{3-4} = 2.9$ Hz, $J_{3-5} = 14$ Hz, H_3), 2.3-2.27 (m, 2H), 1.9-1.85 (m, 2H), 1.42 (s, 2H, *t*-butyl), 1.38 (s, 1H *t*-butyl), 1.34 (s, 4H, *t*-butyl), 1.29 (s, 2H, *t*-butyl)

^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 171.2, 164.8, 153.6, 153.3, 151.2, 151.1, 139.7, 134.2, 134.0, 131.4, 125.9, 125.5, 122.2, 122.0, 121.6, 114.0, 113.9, 79.6, 79.4, 60.5, 59.0, 58.9, 56.4, 56.2, 52.9, 46.9, 46.7, 46.2, 38.7, 31.0, 29.9, 28.6, 28.3, 28.2, 24.4, 23.5

IR ν_{max} (ATR): 1749 cm^{-1} (β -lactam C=O), 1694 cm^{-1} (*N*-Boc-L-proline C=O).

HRMS: APCI calculated for $\text{C}_{29}\text{H}_{36}\text{N}_2\text{NaO}_9$ [**M** + **Na**], 579.2321303 found 579.231643, error + 0.6 ppm

1-(*Tert*-butyl) 2-(2-methoxy-5-((*S*)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (*S*)-pyrrolidine-1,2-dicarboxylate (125b)

125b was resolved from **125** using LC described in **General Method XII**.

Yield: 100 mg (0.17 mmol) 14%

Appearance: white fluffy dust

Melting Point: 75 °C

R_f: 0.26 (plate development 1), 0.41 (plate development 2), 0.50 (plate development 3) (4:1 TBME:*n*-hexane); plate developed three times

^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ (rotamers present) 7.40 (dd, 0.6H, $J = 2.7$ Hz, $J = 8.4$ Hz, $\text{H}_{2''}$), 7.37 (dd, 0.4H, $J = 2.7$ Hz, $J = 8.4$ Hz, $\text{H}_{2''}$), 7.26 (d, 0.2H, $J = 1.7$ Hz, $\text{H}_{6''}$), 7.20 (d, 0.2H, $J = 1.8$ Hz, $\text{H}_{6''}$), 7.19 (d, 0.65H, $J = 9$ Hz, $\text{H}_{3''}$), 7.16 (d, 0.4H, $J = 9$ Hz, $\text{H}_{3''}$), 7.14 (d, 0.2H, $J = 9$ Hz, $\text{H}_{3''}$), 7.13 (d, 0.45H, $J = 9$ Hz, $\text{H}_{3''}$), 6.52 (s, 1H, $\text{H}_{1' \& 3'}$), 6.51 (s, 1H, $\text{H}_{1' \& 3'}$), 5.17 (m, 1H, $\text{H}_{1''}$), 4.41 (dd, 1H, $^2J_{5-3} = 10.3$ Hz, $^3J_{5-4} = 4$ Hz, H_5), 3.77 (s, 2H), 3.76 (s, 1H), 3.64 (s, 1.5H), 3.63 (s, 1.5H), 3.63 (s, 3H), 3.60 (s, 1.2H), 3.56 (s, 1.8H), 3.35 (m, 1H, H_4) 2.97 (dd, 0.5H, $J_{3-4} = 2.9$ Hz, $J_{3-5} = 14$ Hz, H_3), 2.91 (dd, 0.5H, $J_{3-4} = 2.9$ Hz, $J_{3-5} = 14$ Hz, H_3), 2.3-2.27 (m, 2H),

1.9-1.85 (m, 4H), 1.42 (s, 1H, *t*-butyl), 1.39 (s, 2H *t*-butyl), 1.34 (s, 1H, *t*-butyl), 1.3 (s, 4H, *t*-butyl)

¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.3 164.8, 153.6, 153.7, 151.1, 139.6, 134.1, 131.4, 125.9, 126.0, 122.8, 113.8, 79.6, 60.5, 59.0, 56.5, 56.1, 53.1, 46.7, 46.7, 46.5, 38.6, 36.1, 31.0, 28.6, 28.2, 24.4, 23.2

IR ν_{max} (ATR): 1749 cm⁻¹ (β -lactam C=O), 1695 cm⁻¹ (*N*-Boc-L-proline C=O).

HRMS: APCI calculated for C₂₉H₃₆N₂NaO₉ [**M**+Na⁺], 579.2321301 found 579.231780, error - 0.8 ppm

1-(*Tert*-butyl) 2-(2-methoxy-5-(4-oxo-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (2*S*)-pyrrolidine-1,2-dicarboxylate (127)

127 was synthesised from **30** and *N*-Boc-L-proline using **General Method XII**.

Yield: 770 mg (1.23 mmol) 77%

Appearance: white fluffy dust

Melting Point: 87-90 °C

R_f: 0.06 (2:1 TBME:*n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ (rotamers) 7.31 (m, 2H), 7.24 (dd, 0.5H, *J* = 1.7 Hz, *J* = 8.6 Hz, H_{2'}), 7.2 (d, 0.2H, *J* = 2.5 Hz, H_{6'}), 7.09 (d, 2H *J* = 5 Hz), 7.05- 6.99 (m, 2H), 6.59 (s, 2H, H_{1'&3'}), 4.9 (apparent m, 1H, H₃), 4.60-4.46 (m, 2H, H₄ & H_{1''}), 3.86 (s, 2H), 3.85 (s, 1H), 3.79 (s, 3H), 3.75 (s, 6H, H_{7'&9'}), 3.66-3.41 (m, 2H), 2.41-2.26 (m, 2H), 2.10-1.92 (m, 2H), 1.49 (s, 2H), 1.48 (s, 3H), 1.43 (s, 2H), 1.39 (s, 3H)

¹³C NMR (100 MHz, CDCl₃): δ *not determined*

IR ν_{max} (ATR): 1753 cm⁻¹ (β -lactam C=O), 1695 cm⁻¹ (*N*-Boc-L-proline C=O)

HRMS: APCI calculated for C₂₉H₃₆N₂NaO₉ [**M** +Na⁺], 579.2321301 found 579.231780, error - 0.8 ppm

1-(*Tert*-butyl) 2-(2-methoxy-5-((2*S*,3*R*)-4-oxo-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (*S*)-pyrrolidine-1,2-dicarboxylate (127a)

127a was resolved from **127** using LC as described in **General Method XII**.

Yield recovered from diastereomer mixture: 105 mg (0.17 mmol) 14 %

Appearance: yellow/white fluffy powder

Melting Point: 85 °C

R_f: 0.06 (plate development 1), 0.19 (plate development 1), 0.39 (plate development 1), 0.46 (plate development 1), (2:1 TBME:*n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ 7.31 (m, 2H), 7.25 (d, 0.4H, *J* = 2.2 Hz, H_{6'}), 7.24 (d, 0.4H, *J* = 2.2 Hz, H_{6'}), 7.10-6.99 (m, 4H), 6.59 (s, 2H, H_{1'&3'}), 4.90 (d, *J* = 2.3 Hz, 0.6H, H₃), 4.89 (d, *J* = 2.3 Hz, 0.4H, H₃), 4.6-4.47 (m, 2H, H_{1''} & H₄), 3.86 (s, 2H, H_{10'}), 3.85 (s, 1H, H_{10'}), 3.79 (s, 3H, H_{8'}), 3.75 (s, 6H, H_{7'&9'}), 2.41-2.25 (m, 2H), 2.09-1.88 (m, 2H) 1.49 (s, 3H, H_{10''}), 1.43 (6H, *t*-butyl, H_{9''&11''})

¹³C NMR (100 MHz, CDCl₃): δ 170.9, 170.8, 153.6, 153.1, 135.9, 133.6, 129.5, 127.4, 125.8, 125.4, 121.4, 120.3, 113.2, 123.7, 94.9, 80.1, 79.9, 64.2, 60.9, 60.3, 60.1, 59.9, 58.7, 56.1, 46.7, 46.4, 30.9, 29.9, 28.5, 24.4, 23.6

IR ν_{max} (ATR): 1753 cm⁻¹ (β -lactam C=O), 1694 cm⁻¹ (*N*-Boc-L-proline C=O)

HRMS: APCI calculated for C₃₃H₃₇N₂O₉S [**M**-H⁺]; *m/z* 637.222525; found 637.222326, error - 0.3 ppm

1-(*Tert*-butyl 2-(2-methoxy-5-((2*R*,3*S*)-4-oxo-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (*S*)-pyrrolidine-1,2-dicarboxylate (127b)

127b was resolved from **127** using LC as described in **General Method XII**.

Yield recovered from diastereomer mixture: 200 mg (0.32 mmol) 20 %

Appearance: yellow/white fluffy powder

Melting Point: 83 °C

R_f: 0.06 (plate development 1), 0.19 (plate development 1), 0.29 (plate development 1), 0.41 (plate development 1), (2:1 TBME:*n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ 7.31 (m, 1H), 7.24 (dd, 0.3H, *J* = 2.2 Hz, *J* = 7.9 Hz, H_{2''}), 7.21 (d, 0.3H, *J* = 2.2 Hz, H_{6''}), 7.09 (m, 2H), 7.05 (m, 2H), 6.56 (s, 2H, H_{1'&3'}), 4.91 (d, 0.6H, *J* = 2.7 Hz, H₃), 4.9 (d, 0.4H, *J* = 2.2 Hz, H₃), 4.61-4.5 (m, 3H, H₄ & H_{1'''}), 3.86 (s, 2H, H_{10'}), 3.85 (s, 1H, H_{10'}), 3.79 (s, 3H, H₈), 3.75 (s, 6H, H_{7'&9'}), 3.66-3.42 (m, 2H), 2.42 – 2.2 (m, 2H), 2.1-1.9 (m, 2H), 1.47 (s, 3H, H_{10'''}), 1.40 (s, 6H, H_{9'''&11'''})

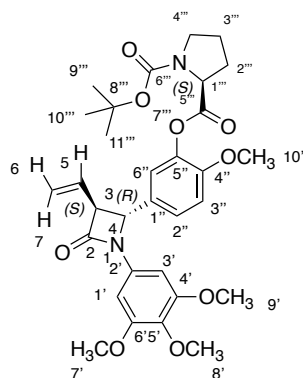
¹³C NMR (100 MHz, CDCl₃): δ 170.9, 170.81, 164.3, 164.1, 154.5, 153.9, 153.6, 151.6, 140.4, 135.9, 134.8, 134.6, 133.5, 129.6, 129.5, 127.5, 127.3, 125.9, 125.4, 124.5, 124.1, 121.3, 120.4, 120.2, 113.2, 95.0, 80.1, 80.0, 64.2, 60.9, 60.3, 59.1, 56.1, 46.2, 46.4, 31.1, 30.1, 28.4, 28.3, 27.0, 24.4, 23.5

IR ν_{max}(ATR): 1754 cm⁻¹ (β-lactam C=O), 1694 cm⁻¹ (*N*-Boc-L-proline C=O)

HRMS: APCI calculated for C₃₃H₃₇N₂O₉S [**M-H**⁺]; *m/z* 637.222525; found 637.222556, error - 0.0 ppm

1-(*Tert*-butyl 2-(2-methoxy-5-(4-oxo-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-yl)phenyl) (*2S*)-pyrrolidine-1,2-dicarboxylate (128)

128 was synthesised from **33** and *N*-Boc-L-proline using **General Method XII**.



Yield: 310 mg (0.515 mmol) 43%

R_f: 0.1 (2: 1 TBME: *n*-hexane)

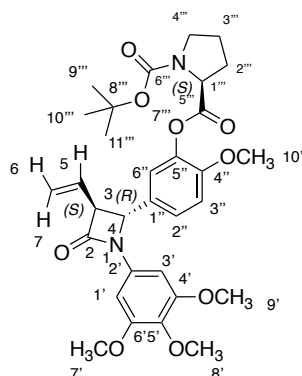
¹H NMR (400 MHz, CDCl₃): δ 7.24 (dd, 0.2H, *J*_{2''-6''} = 2.5 Hz, *J*_{2''-3''} = 9.1 Hz, H_{2''}), 7.23 (d, 0.4H, *J*_{6''-2''} = 2.5 Hz, H_{6''}), 7.18 (dd, 0.8H, *J*_{2''-6''} = 2.5 Hz, *J*_{2''-3''} = 9.1 Hz, H_{2''}), 7.03 (d, 0.6H, *J*_{6''-2''} = 2.5 Hz, H_{7''}), 7.00 (d, 0.6H, *J*_{3''-2''} = 9.1 Hz, H_{3''}), 6.97 (d, 0.4H, *J*_{3''-2''} = 9.1 Hz, H_{3''}), 6.54 (s, 2H, H_{1'&3'}), 6.01 (apparent m, 1H, H₅), 5.40 (apparent doublet, 1H, *J*₇₋₅ = 16 Hz, H₇), 5.35 (apparent doublet, 1H *J*₆₋₅ = 10 Hz, H₆), 4.73 (d, 0.6H, *J*₄₋₃ = 2.2 Hz, H₄), 4.70 (d, 0.4H, *J*₄₋₃ = 2.2 Hz, H₄), 4.57 (m, 0.4H, H_{1'''}), 4.46 (m, 0.6H, H_{1'''}), 3.84 (s, 2H), 3.83 (s, 1H), 3.77 (s, 3H), 3.73 (apparent s, 7H, H_{7'&9'} & H₃), 3.66-3.42 (m, 2H), 2.43-2.25 (m, 3H), 2.1 – 1.9 (m, 2H), (1.49 (s, 1.5H), 1.45 (s, 2.5H), 1.44 (s, 2H), 1.39 (s, 3H)

¹³C NMR (100 MHz, CDCl₃): δ 170.9, 165.1, 153.7, 151.3, 140.3, 134.5, 133.7, 130.3, 129.9, 124.5, 123.9, 120.1, 113.1, 94.7, 80.1, 63.9, 61.0, 59.1, 56.1, 46.8, 46.4, 38.9, 31.1, 30.2, 28.5, 24.4, 23.7

HRMS: APCI calculated for C₃₁H₃₇N₂O₉ [**M-H**⁺]; m/z 581.250454; found 581.250273, error + 0.3 ppm

1-(*Tert*-butyl) 2-(2-methoxy-5-((2*S*,3*R*)-4-oxo-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-yl)phenyl) (*S*)-pyrrolidine-1,2-dicarboxylate (128a)

128a was resolved from **128** using LC as described in **General Method XII**.



Yield resolved from diastereomer mixture: 120mg (0.21 mmol) 40 %

R_f: 0.1 (plate development 1), 0.18 (plate development 1), 0.25 (plate development 1), 0.35 (plate development 1), 0.45 (plate development 1), (2: 1 TBME: *n*-hexane).

¹H NMR (400 MHz, CDCl₃): δ 7.31 (dd, 0.3H, *J*_{2''-6''} = 2.8 Hz, *J*_{2''-3''} = 8.6 Hz, H_{2'}), 7.24 (dd, 0.3H, *J*_{2''-6''} = 2.8 Hz, *J*_{2''-3''} = 8.6 Hz, H_{2'}), 7.21 (d, 0.2H, *J*_{6''-2''} = 2.5 Hz, H_{6''}), 7.19 (d, 0.3H, *J*_{6''-2''} = 2.5 Hz, H_{6''}), 7.03 (d, 0.4H, *J*_{6''-2''} = 2.5 Hz, H_{6''}), 7.01 (d, 0.5H, *J*_{3''-2''} = 8.5 Hz, H_{3''}), 6.98 (d, 0.5H, *J*_{3''-2''} = 8.5 Hz, H_{3''}), 6.59 (s, 0.35H, H_{1'&3'}), 6.55 (s, 1.6H, H_{1'&3'}), 6.03 (apparent m, 1H, H₅), 5.40 (apparent doublet, 1H, *J*₇₋₅ = 17.1 Hz, H₇), 5.34 (apparent doublet, 1H, *J*₆₋₅ = 10.2 Hz, H₆), 4.73 (d, 0.5H, *J*₄₋₃ = 2.5 Hz, H₄), 4.7 (d, 0.4H, *J*₄₋₃ = 2.5 Hz, H₄), 4.59 (m, 0.5H, H_{1'''}), 4.48 (m, 0.5H, H_{1'''}), 3.86 (s, 1H), 3.84 (s, 1H), 3.83 (s, 1H), 3.77 (s, 3H), 3.75 (s, 1H, H_{7'&9'}), 3.73 (s, 5H, H_{7'&9'}), 3.66-3.42 (m, 3H, Proline CH₂), 2.4-2.25 (m, 2H, Proline CH₂), 2.1-2.0 (m, 1H, Proline CH₂), 1.99 (dd, 1H, *J*₃₋₁ = 2.5 Hz, *J*₃₋₅ = 7.2 Hz, H₃), 1.4 (s, 6H, *t*-butyl), 1.49 (s, 3H, *t*-butyl)

¹³C NMR (100 MHz, CDCl₃): δ 170.8, 170.7, 165.23, 164.0, 163.8, 153.6, 151.4, 147.3, 142.3, 140.1, 133.7, 130.3, 129.8, 129.6, 128.3, 127.2, 125.2, 124.4, 124.1, 123.9, 122.2, 121.5, 120.4, 120.1, 120.2, 113.0, 112.9, 112.2, 94.7, 94.5, 80.2, 80.0, 63.9, 63.7, 62.7, 61.6, 60.97, 59.1, 58.8, 56.1, 56.0, 46.7, 46.4, 31.0, 30.0, 28.5, 28.3, 27.0, 24.3, 23.5, 18.3

HRMS: APCI calculated for C₃₁H₃₇N₂O₉ [**M-H**⁺]; m/z 581.250454; found 581.250145, error + 0.3 ppm

1-(*Tert*-butyl) 2-(2-methoxy-5-((2*R*,3*R*)-4-oxo-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-yl)phenyl) (*S*)-pyrrolidine-1,2-dicarboxylate (128b)

128b was resolved from **128** using LC as described in **General Method XII**.

Yield: 100 mg (0.17 mmol) 33 %

R_f: 0.1 (plate development 1), 0.18 (plate development 1), 0.25 (plate development 1), 0.30 (plate development 1), 0.402 (plate development 1), (2: 1 TBME: *n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ 7.24 (dd, 0.4H, $J_{2''-6''} = 1.6$ Hz, $J_{2''-3''} = 8.5$ Hz, H_{2''}), 7.21 (dd, 0.6H, $J_{2''-6''} = 1.6$ Hz, $J_{2''-3''} = 8.5$ Hz, H_{2''}), 7.16 (d, 0.4H, $J_{6''-2''} = 2$ Hz, H_{6''}), 7.03 (d, 0.6H, $J_{6''-2''} = 2$ Hz, H_{6''}), 7.0 (d, 0.5H, $J_{3''-2''} = 8$ Hz, H_{3''}), 6.97 (d, 0.5H, $J_{3''-2''} = 8$ Hz, H_{3''}), 6.58 (s, 0.2H, H_{1'&3'}), 6.55 (s, 1.8H, H_{1'&3'}), 6.02 (apparent m, 1H, H₅), 5.39 (d, $J_{7-5} = 17.4$ Hz), 5.34 (d, 1H, $J_{6-5} = 10.1$ Hz), 4.73 (d, 0.5H, $J_{4-3} = 2.5$ Hz, H₄), 4.71 (d, 0.4H, $J_{4-3} = 2.5$ Hz, H₄), 4.58 (m, 0.4H, H_{1'''}), 4.48 (m, 0.6H, H_{1'''}), 3.84 (s, 2H, H_{8'}), 3.83 (s, 1H, H_{8'}), 3.78 (s, 3H, H_{10'}), 3.74 (s, 6H, H_{7'&9'}), 3.66-3.42 (m, 2H), 2.42-2.24 (m, 2H), 2.14-1.19 (m, 2H), 1.48 (s, 3H, H_{10'''}), 1.4 (s, 6H, H_{9'''&11'''})

¹³C NMR (100 MHz, CDCl₃): δ 170.9, 170.7, 165.6, 153.6, 151.4, 140.2, 133.7, 130.4, 129.9, 129.8, 124.4, 124.0, 120.2, 119.9, 113.0, 94.7, 80.0, 79.93, 64.0, 63.7, 60.9, 59.0, 56.1, 55.9, 46.6, 46.3, 38.7, 31.0, 30.0, 28.4, 28.3, 25.7, 24.4, 23.6

HRMS: APCI calculated for C₃₁H₃₇N₂O₉ [**M-H**⁺]; *m/z* 581.250454; found 581.250273, error + 0.3 ppm

1-(*Tert*-butyl) 2-(2-methoxy-5-(4-oxo-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (*S*)-pyrrolidine-1,2-dicarboxylate (129**)**

129 was synthesised using **General Method XII** from **38** and *N*-Boc-L-proline.

Yield: 70 mg (0.11 mmol) 11%

Appearance: white fluffy dust

Melting Point: 64-66 °C

R_f: 0.22 (1:1 *n*-hexane: ethyl acetate). 0.15 (plate development 1), 0.3 (plate development 2), 0.5 (plate development 3), (1:1 TBME:*n*-hexane)

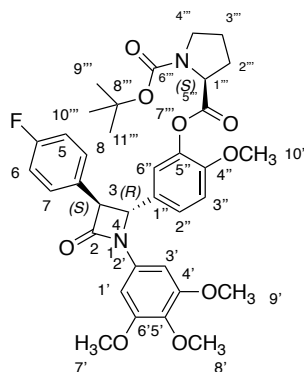
¹H NMR (400 MHz, CDCl₃): δ 7.24 (m, 2H), 7.08-6.86 (m, 6H), 6.57 (s, 1H, H_{1'&3'}), 6.54 (s, 1H, H_{1'&3'}), 5.1 (apparent s, 1H, H₃), 4.89 (m, 1H, H₄), 4.59- 4.85 (m, 1H, H_{1'''}), 3.93 (s, 1H), 3.85 (s, 2H), 3.75 (s, 3H), 3.71 (s, 3H, H_{7'&9'}), 3.70 (s, H_{7'&9'}), 3.66-3.99 (m, 2H), 2.41-1.90 (m, 2H, CH₂), 1.48-1.36 (multiple s, 9H, *t*-butoxy, H_{14'-16'})

¹³C NMR (400 MHz, CDCl₃): δ 170.9, 162.6, 157.1, 153.4, 151.9, 130.1, 129.7, 122.3, 121.5, 118.5, 115.6, 112.4, 95.6, 95.2, 87.7, 63.5, 56.3, 61.0, 59.0, 56.5, 46.7, 46.4, 31.0, 30.0, 28.6, 28.2

IR ν_{max} (ATR): 1760 cm⁻¹ (β -lactam C=O), 1694 cm⁻¹ (*N*-Boc- L-proline C=O)

HRMS: APCI calculated for C₃₀H₃₃N₂N₂O₈ [**M +H**⁺], 549.223142, found 549.222837, error - 0.6 ppm, (mass and formula for 21DSMix without *t*-butyl group)

1-(*Tert*-butyl) 2-(5-(3-(4-fluorophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)-2-methoxyphenyl) (2*S*)-pyrrolidine-1,2-dicarboxylate (130**)**



130 was synthesised from **39** and *N*-Boc-L-Proline using **General Method XII**.

Yield: 470mg (0.72 mmol) 37%

Appearance: white/yellow fluffy dust

Melting Point: 79-83 °C

R_f: 0.23 (2:1 TBME:*n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ 7.32 (apparent dd, 2H, *J* = 4 Hz, *J* = 3.8 Hz, H_{5&6'}), 7.25 (dd, 0.5H, *J* = 6.7 Hz, *J* = 1.5 Hz, H_{2''}), 7.20 (dd, 0.5H, *J* = 12.5 Hz, *J* = 1.8 Hz, H_{2''}), 7.11 – 6.9 (m, 4H, H_{7&8}, H_{3''}, H_{6''}), 6.60 (s, 2H, H_{1'&3'}), 4.82 (apparent m, 1H, H₃), 4.57 (m, 0.5H, H_{1''}), 4.48 (m, 0.5H, H_{1''}), 4.32 (d, 0.15H, *J* = 2.4 Hz, H₃), 4.29 (d, 0.4H, *J* = 2.4 Hz, H₃), 4.27 (apparent s, 1H, 0.5H, H₄), 3.86 (s, 2H), 3.85 (s, 2H), 3.79 (s, 3H), 3.75 (s, 6H), 3.67-3.42 (m, 2H), 2.34-2.19 (m, 2H), 2.11-1.96 (m, 2H), (1.49 (s, 1.5H), 1.47 (s, 3H), 1.43 (s, 1.5H), 1.39 (s, 3H)

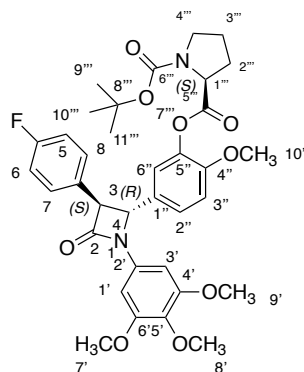
¹³C NMR (100 MHz, CDCl₃): not obtained due to low sample concentration

¹⁹F NMR (376 MHz, CDCl₃): δ -113.82 (major peak), -113.95 (minor peak) -114.07 (minor peak), -114.05 (main peak), -114.4 (minor peak)

IR ν_{max} (ATR): 1749 cm⁻¹ (β-lactam C=O), 1639 cm⁻¹ (*N*-Boc-proline C=O)

HRMS: APCI calculated for C₂₉H₃₆N₂NaO₉ [**M**+Na⁺], 579.2321301 found 575.231780, error - 0.8 ppm

1-(*Tert*-butyl) 2-(5-((2*S*,3*R*)-3-(4-fluorophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)-2-methoxyphenyl) (*S*)-pyrrolidine-1,2-dicarboxylate (130a**)**



130a was resolved from **130** using LC conditions described in **General Method XII**.

Yield resolved from diastereomer mixture: 50mg (0.08 mmol) 11%

Appearance: white fluffy dust

R_f: 0.13 (plate development 1), 0.2 (plate development 2), 0.31 (plate development 3), (2:1 TBME:*n*-hexane)

¹H NMR (600 MHz, CDCl₃): δ 7.32 (dd, 2H, *J* = 8 Hz, *J* = 3.6 Hz, H_{5&6}), 7.22 (d, 0.6H, *J* = 1.8 Hz, H_{6''}), 7.09 (apparent triplet, 2H, *J* = 8 Hz, H_{7&8}), 7.06 (d, 0.6H, *J* = 1.8 Hz, H_{6''}), 7.03 (d, 0.5H, *J* = 8.8 Hz, H_{3''}), 7.00 (d, 0.6H, *J* = 8 Hz), 6.61 (s, 0.4H, H_{1'&3'}), 6.58 (s, 1.6H, H_{1'&3'}), 5.43 (apparent s, 0.4H, H₃), 5.34 (apparent s, 0.5H, H₃), 4.94 (apparent s, 0.5H, H₄), 4.81 (apparent s, 0.45H, H₄), 4.31 (m, 1H, H₄), 3.83 (s, 1.5H, H_{8'}), 3.82 (s, 1.5H, H_{8'}), 3.78 (s, 3H, H_{10'}), 3.72 (s, 3H, H_{7'&9'}), 3.58 – 3.78 (m, 2H), 2.36-2.14 (m, 2H), 2.12 – 1.86 (m, 2H), 1.51 (s, 6H, H_{9''&11''}), 1.46 (s, 3H, H_{10''})

¹³C NMR (100 MHz, CDCl₃): δ 172.4, 171.9, 161.6, 158.8, 158.2, 153.5, 153.4, 133.1, 128.6, 128.4, 126.6, 125.1, 110.23, 110.1, 95.6, 95.4, 82.8, 80.2, 80.1, 63.8, 61.0, 59.3, 58.6, 56.1, 55.4, 46.7, 46.5, 46.5, 46.4, 29.9, 28.4, 27.0, 24.5, 23.6, 16.3

¹⁹F NMR (376 MHz, CDCl₃): δ -113.8 (major peak), -113.9 (minor peak), -114.0 (major peak).

HRMS: APCI calculated for C₂₉H₃₆N₂NaO₉ [**M**+Na⁺], 579.2321301 found 575.231780, error - 0.8 ppm

1-(*Tert*-butyl) 2-(5-((2*R*,3*S*)-3-(4-fluorophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)-2-methoxyphenyl) (*S*)-pyrrolidine-1,2-dicarboxylate (130b)

130b was resolved from **130** using LC described in **General Method XIII**.

Yield resolved from diastereomer mixture: 70mg (0.11 mmol) 15%

Appearance: white fluffy dust

R_f: 0.13 (plate development 1), 0.13 (plate development 1), 0.28 (plate development 1), (2:1 TBME:*n*-hexane);

¹H NMR (400 MHz, CDCl₃): δ 7.32 (dd, 2H, *J* = 7.1 Hz, *J* = 3.7 Hz), 7.24 (m, 1H), 7.09 (m, 3H), 7.02 (apparent triplet, *J* = 8.6 Hz), 6.59 (s, 2H, H_{1'&3'}), 4.82 (apparent m, 1H, H₃), 4.57 (m, 0.5H, H_{1''}), 4.49 (m, 0.5H, H_{1'''}), 4.32 (apparent s, 0.2H, H₄), 4.29 (apparent s, 0.3H, H₄), 4.28 (apparent s, 0.6H, H₄), 3.86 (s, 2H, H_{8'}), 3.85 (s, 1H, H_{8'}), 3.79 (s, 3H, H_{10'}), 3.75 (s, 6H, H_{1'&3'}), 3.68 – 3.42 (m, 2H), 2.42– 1.98 (m, 4H), 1.49– 1.35 (apparent m, 9H, H_{9''',10''',11'''})

¹³C NMR (100 MHz, CDCl₃): not obtained due to low sample concentration

¹⁹F NMR (376 MHz, CDCl₃): δ -113.82 (major peak), -114.03 (minor peak), -114.09 (major peak), -114.4 (minor peak)

HRMS: APCI calculated for C₂₉H₃₆N₂NaO₉ [**M**+Na⁺], 579.2321301 found 575.231780, error - 0.8 ppm

1-(*tert*-butyl) 2-(2-methoxy-5-(3-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (*2S*)-pyrrolidine-1,2-dicarboxylate (131)

131 was synthesised from **41** and *N*-Boc- L -proline using **General Method XII**.

Yield: 500 mg (0.76 mmol) 35%

Appearance: white fluffy dust

Melting Point: 98-103 °C

R_f: 0.2 (2:1 TBME:*n*-hexane)

¹H NMR (400 MHz, CDCl₃): δ (rotamers) 7.26 (d, 3H, *J* = 8.1), 7.21 (d, 0.14H, *J* = 2 Hz, 0.14H, H_{6''}), 7.18 (d, 0.3H, *J* = 2 Hz, H_{6''}), 7.06 (d, 0.6H, *J* = 2 Hz, H_{6''}), 7.01 (d, 1H, *J* = 8.4 Hz, H_{3''}), 6.92 (apparent m, 2H), 6.61 (s, 2H, H_{1'&3'}), 4.8 (m, 1H, H₃), 4.58 (m, 0.4H, H_{1''}), 4.49 (m, 0.6H, H_{1'''}), 4.29 (d, 0.15H, *J* = 2.3 Hz, H₄, rotamer 1), 4.26 (d, 0.3H, *J* = 2.3 Hz, H₄, rotamer 2), 4.23 (apparent s, 0.6H, H₄, rotamer 3), 3.85 (s, 1H, H_{8'}, rotamer 1), 3.84 (s, 2H, H_{8'}, rotamer 2), 3.83 (s, 1H, H_{10'}), 3.79 (s, 2H, H_{10'}), 3.75 (s, 6H, H_{7'&9'}), 3.68-3.42 (m, 2H), 2.41 – 1.92 (m, 4H), 1.49 (s, 2H), 1.47 (s, 2H) 1.43 (s, 2H), 1.39 (s, 3H).

¹³C NMR (100 MHz, CDCl₃): δ 170.9, 165.9, 165.8, 159.4, 159.3, 153.6, 151.4, 140.3, 140.2, 133.7, 130.1, 126.7, 126.6, 124.4, 121.5, 121.3, 120.4, 113.2, 113.1, 80.1, 80.0, 79.9, 64.5, 64.4, 63.8, 63.8, 63.8, 60.9, 59.1, 58.8, 56.1, 55.9, 55.3, 56.6, 46.4, 38.7, 37.2, 31.1, 30.1, 30.0, 28.4, 28.3, 28.3, 27.0, 24.4, 24.3, 23.6

IR ν_{max} (ATR): 1749 cm⁻¹ (β-lactam C=O), 1694 cm⁻¹ (*N*-Boc-L-proline C=O)

1-(*tert*-butyl) 2-(2-methoxy-5-((2*S*,3*R*)-3-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (*S*)-pyrrolidine-1,2-dicarboxylate (131a)

131a was resolved from the mixture of **131** using the column chromatography conditions described in **General Method XII**.

Yield: 90 mg (0.14 mmol) 18 %

Appearance: white fluffy dust

Melting Point: 101-103 °C

R_f: 0.4 (4:1 TBME:*n*-hexane) plate developed twice

¹H NMR (400 MHz, CDCl₃): δ (rotamers present) 7.26 (d, *J* = 8.1 Hz, 3H), 7.21 (d, 0.45H, *J* = 2 Hz, H_{6''}), 7.06 (d, 1H, *J* = 2 Hz, H_{6''}), 7.01 (apparent triplet, 1H, *J* = 8.4 Hz), 6.93 (d, 2H, *J* = 9 Hz), 6.61 (s, 2H, H_{1'&3'}), 4.8 (d, *J* = 2.3 Hz, 1H, H₃), 4.59 (m, 0.5H, H_{1''}), 4.48 (m, 0.5H, H_{1''}), 4.29 (d, 0.3H, *J* = 2.3 Hz, H₄), 4.24 (d, 0.7H, *J* = 2.3 Hz, H₄), 3.85 (s, 2H, H_{8'}), 3.84 (s, 1H, H_{8'}), 3.83 (s, 3H), 3.79 (s, 3H), 3.75 (s, 6H, H_{7'&9'}), 3.67-3.43 (m, 2H), 2.41 – 1.9 (m, 4H), 1.49 (s, 3H, H_{10''}), 1.43 (s, 6H, H_{9''&11''})

¹³C NMR (100 MHz, CDCl₃): δ 170.8, 170.0, 165.9 (C₂), 165.8 (C₂), 159.4, 153.8 (C_{4'&6'}), 153.4 (C_{4'&6'}), 151.4, 151.3, 151.3, 140.2, 133.7, 133.1, 128.7, 126.5, 124.4, 124.1, 121.5, 120.4, 114.5, 113.2, 113.1, 94.8 (C_{1'&3'}), 80.1, 80.0, 79.9, 64.6, 64.4, 63.9, 60.9, 59.0, 58.8, 56.1, 55.9, 55.4, 46.61, 46.4, 31.0, 30.1, 28.5, 28.4, 24.3, 23.6

HRMS: APCI calculated for C₃₆H₄₁N₂O₁₀ [**M-H**⁺]; 661.27669; found 661.277374, error + 1.1 ppm

1-(*tert*-butyl) 2-(2-methoxy-5-((2*R*,3*S*)-3-(4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) (*S*)-pyrrolidine-1,2-dicarboxylate (131b)

131b was resolved from the mixture of **131** using the LC as described in **General Method XII**.

Yield: 110 mg (0.17 mmol) 23% **Appearance:** white fluffy dust

Melting Point: 100-103 °C

R_f: 0.38 (2:1 TBME:*n*-hexane), plate developed twice

¹H NMR (400 MHz, CDCl₃): δ 7.26 (d, 3H, *J* = 8.1 Hz), 7.10 (d, 0.14H, *J* = 2 Hz, H_{3''}), 7.19 (d, 0.3H, *J* = 2 Hz, H_{3''}), 7.07 (d, 0.6H, *J* = 2 Hz, H_{3''}), 7.02 (d, 1H, *J* = 8.4 Hz, H_{2''}), 6.92 (apparent m, 2H), 6.6 (s, 2H, H_{1'&3'}), 4.8 (m, 0.85, H₃), 4.7 (d, 0.15H, *J* = 2.3 Hz, H₃), 4.57 (m, 0.45H, H_{1''}), 4.49 (m, 0.6H, H_{1''}), 4.28 (d, 0.15H, *J* = 2.3 Hz, H₄), 4.26 (d, 0.3H, *J* = 2.3 Hz, H₄), 4.24 (apparent s, 0.6H, H₄), 3.85 (s, 2H, H_{8'}), 3.84 (s, 2H, H_{8'}), 3.83 (s, 1H, 3-Ph-OCH₃), 3.79 (s, 3H, H_{10''}), 3.74 (s, 6H, H_{7'&9'}), 3.67-3.43 (m, 2H), 2.42 – 1.93 (m, 4H), 1.49 (s, 1H), 1.47 (s, 2H), 1.43 (s, 1H), 1.39 (s, 3H)

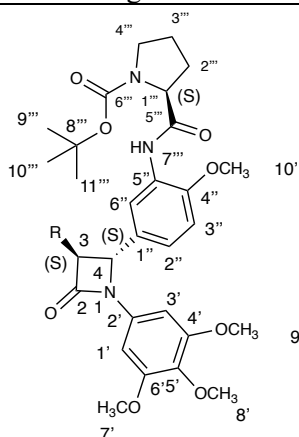
¹³C NMR (100 MHz, CDCl₃): not obtained due to low sample concentration

HRMS: APCI calculated for C₃₆H₄₁N₂O₁₀ [**M-H**⁺]; 661.27669; found 661.276838, error + 1.1 ppm

General Method XIII: Coupling of *N*-Boc-L-proline to NH₂ to form β-lactam diastereomers using HOBt and DCC

To a stirring solution of amino substituted β-lactam (1 eq, 0.23 mmol) in 10 mL of DMF under a nitrogenous atmosphere, HOBt (1.6 eq, 0.36 mmol, 50mg) and DCC (1.2 eq, 0.28 mmol, 57 mg) were added. *N*-(Boc)-L-Proline (1.2 eq, 0.28 mmol, 60mg) was then added to the reaction vessel. The reaction was stirred for 24 hours and subsequently diluted with ethyl acetate (40 mL) and filtered to remove the DCC prior to work up. The organic filtrate was washed with dH₂O (3 X 30 mL) to remove residual DMF, followed by 30 mL of NaHCO_{3(sat)} solution to remove excess proline. The organic layer was dried with anhydrous Na₂SO₄, filtered and solvent removed *in vacuo* to afford a crude yellow gel. The crude mixture was purified using flash chromatography over silica gel using isocratic conditions of 1: 1 *n*-hexane: ethyl acetate. TLC using various ratios of TBME: *n*-hexane failed to achieve separation of amide diastereomer derivatives. No further diastereomer purification was carried out.

Numbering nomenclature for B ring *meta* derivatised amide diastereomers



Diastereomer Code	R
137	
138	

Tert-butyl-(*S*)-2-((2-methoxy-5-(4-oxo-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (137)

137 was synthesised from **37** *trans* and *N*-Boc-L-proline using **General Method XIII**.

Yield: *not calculated*

Appearance: brown oil

R_f: 0.18 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 7.25 (m, 2H), 7.01 (t, 1H *J* = 7.6 Hz), 6.93 (m, 4H), 6.63 (s, 1.5H, H_{7'&9'}), 6.62 (s, 0.5H, H_{7'&9'}), 5.18 (apparent s, 1H, H₄), 4.97 (apparent s, 1H, H₃), 4.37 (m, 1H, H_{1'''}), 3.91 (s, 3H, H), 3.79 (s, 3H), 3.74 (s, 6H, H_{7'&9'}), 3.67-3.44 (m, 2H, proline CH₂), 2.42-1.89 (m, 4H, proline CH₂), 1.51 (s, 6H, H_{9'''&11'''}), 1.45 (s, 3H, H_{10'''})

¹³C NMR (100 MHz, CDCl₃): δ 173.9, 162.7, 157.1, 153.4, 134.8, 133.2, 129.7, 129.3, 126.6, 122.4, 121.5, 118.6, 117.5, 114.8, 114.4, 111.2, 110.8, 95.7, 87.2, 81.9, 80.8, 64.2, 61.1, 59.4, 59.0, 56.2, 55.9, 47.3, 46.6, 30.9, 28.2, 24.2, 23.7

HRMS: APCI calculated for C₃₅H₄₀N₃O₉ [**M-H**⁺]; 646.277003; found 646.278552, error -2.4 ppm

Tert-butyl(*R*)-2-((2-methoxy-5-4-oxo-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (138)

138 was synthesised from **32** *trans* and *N*-Boc-L-proline using both **General Method XII** and **General Method XIII** with good agreement of spectroscopic data using both methods.

Yield General Method XII: 30 mg (0.048 mmol) 20%.

Yield General Method XIII: 50 mg (0.08 mmol) 35 %

R_f: 0.39 (1:1 *n*-hexane: ethyl acetate), 0.11 (2:1 *n*-hexane: TBME).

Melting Point: 65-68 °C

¹H NMR (600 MHz, CDCl₃): δ 9.29 (bs, NH, H_{7'''}), 8.58 (bs, 1H), 7.29 (d, 1H, *J* = 4.5 Hz), 7.1 (bs, 1H), 7.08 (apparent s, 1H), 7.02 (t, 1H, *J* = 5.9 Hz), 6.91 (bs, 1H), 6.62 (2H, H_{1'&3'}), 4.93

(apparent s, 1H, H₃), 4.53 (apparent s, 1H, H₄), 4.51-4.28 (m, 1H, H₁'), 3.89 (s, 3H, H₁₀'), 3.78 (s, 3H, H₈'), 3.75 (s, 6H, H_{7&9}'), 3.66-3.39 (broad m, 2H, proline CH₂), 1.96-1.62 (broad m, 4H, proline CH₂), 1.55-1.32 (apparent m, 9H, H_{9''-11''})

¹³C NMR (100 MHz, CDCl₃): δ 170.5, 164.5, 153.4, 148.3, 136.1, 134.5, 133.7, 127.2, 125.9, 125.2, 118.3, 110.7, 110.4, 97.7, 95.2, 95.0, 80.6, 72.7, 65.8, 64.8, 60.9, 60.1, 56.1, 55.9, 49.3, 47.2, 33.8, 28.3, 25.5, 24.9

¹⁵N NMR (60.8 MHz, CDCl₃): δ 117.8 (NH, H₇')

IR ν_{max} (ATR): 1752 cm⁻¹ (β-lactam C=O), 1690 cm⁻¹ (N-Boc-L-proline C=O)

HRMS: APCI calculated for C₃₃H₃₈N₃O₈S [**M-H**⁺]; 636.238510; found 636.238493, error -0.0 ppm

General Method XIV: Hydrazinolysis of N-Boc-L-proline from β-lactam diastereomers to afford optically pure enantiomers

Hydrazine dihydrochloride (5 eq) was added to a stirring solution of the respective diastereomer (1 eq) in anhydrous methanol (30 mL) at 0 °C (on ice) under a nitrogenous atmosphere. Anhydrous TEA (9 eq) was added dropwise to the vessel. The solution was allowed reach room temperature before heating to reflux for 6 hours. The temperature was maintained at 80 °C. The solvent was removed under reduced pressure and residue treated with a saturated solution of potassium hydrogen sulfate (KHSO₄). Extraction was performed using ethyl acetate (2 × 30 mL). The organic layers were washed with sodium bicarbonate (3 × 20 mL) to ensure removal of proline. The organic phase was dried with anhydrous Na₂SO₄, filtered and solvent removed under reduced pressure. The crude was then purified using flash chromatography over silica gel (eluent: *n*-hexane:ethyl acetate; 1:2) to afford the desired product. Spectroscopic data is in line with racemates for enantiomers listed below as detailed in **Appendix 3**.

(3*S*,4*S*)-3-Hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (16EN1)

16EN1 was synthesised from **116a** and **123a** using **General Method XIV**.

Yield: (116a to 16EN1): 22.5 mg (0.06 mmol) 30%

Yield: (123a to 16EN1): 30mg (0.08 mmol) 49%

Melting Point: 64-68 °C

R_f: 0.2 (1:1; *n*-hexane:ethyl acetate)

HRMS: APCI calculated for [**M + H**⁺], 376.139869; found 376.139078, error + 2.1 ppm

IR ν_{max} (ATR): 3221 cm⁻¹ (OH)·1727 cm⁻¹ (β-lactam C=O)

Purity (RP-HPLC): 97%

Ee: 75%

(3*R*, 4*R*)-3-Hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (16EN2)

16EN2 was synthesised from **116b** and **123b** using **General Method XIV**.

Yield: (116b to 16EN1): 19 mg (0.05 mmol), 31%

Yield: (123b to 16EN1): 30 mg (0.08 mmol) 61%

Melting Point: 69-73 °C

R_f: 0.2 (1:1; n-hexane:ethyl acetate)

IR ν_{max} (ATR): 3225 cm⁻¹ (OH), 1743 cm⁻¹ (β -lactam C=O)

HRMS: APCI calculated for [M+H⁺], 376.139204; found 376.139078, error -0.3 ppm

Purity (RP-HPLC): 90 %

Ee: 41%

***Dextrorotatory*-(3*S*, 4*S*)-3-hydroxy-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (17EN1)**

17EN1 was synthesised from 108a using **General Method XIV**.

Yield: 58mg (0.16 mmol), 30%

Appearance: white powder

Melting Point: 120-122°C

R_f: 0.36 (1:1; n-hexane:ethyl acetate)

[α]_D²⁰: +25.14 (*c* 17.9mg/5mL in chloroform)

IR ν_{max} (ATR): 3284 cm⁻¹ (OH), 1726 cm⁻¹ (β -lactam C=O)

APCI calculated for [M+H⁺], 360.144560; found 360.144164, error -2.1 ppm and [M+ Na⁺], 382.126696; found 382.126108, error -2.1 ppm

Purity (RP-HPLC): 97%

Ee: 94%

***Levorotatory*-(3*R*,4*R*)-3-hydroxy-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (17EN2)**

17EN2 was synthesised from 108b using **General Method XIV**.

Yield: 150mg (0.42mmol) 66%

Appearance: White powder

Melting Point: 122-127 °C

R_f: 0.36 (1:1; *n*-hexane: ethyl acetate)

[α]_D²⁰: -25.38 (*c* 19.7mg/5mL in chloroform)

IR ν_{max} (ATR): 3295.35 cm⁻¹ (OH), 1723.34 cm⁻¹ (β -lactam C=O)

HRMS: APCI calculated for [M +H⁺], 360.144698; found 360.144164, error + 1.5 ppm and [M⁺+ Na], 382.126695; found 382.126108, error + 1.5 ppm

Purity (RP-HPLC): 91%

Ee: 74%

***Dextrorotatory*-(3*S*,4*S*)-4-(3-fluoro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (18EN1)**

18EN1 was synthesised from 114a using **General Method IX**.

Yield: 90mg, (0.24mmol) 46%

Appearance: white powder

Melting Point: 143-146 °C

R_f: 0.25 (1:1 *n*-hexane:ethyl acetate)

[α]_D²⁰: +22.15 (*c* 15.8mg/5mL in chloroform)

IR ν_{max} (ATR): 3288 cm⁻¹ (OH), 1726 cm⁻¹ (β -lactam C=O)

HRMS: APCI calculated for [M +H⁺], 378.134910; found 378.134742, error +0.4 ppm

Purity (HPLC): 95%

Ee: 79%

Levorotatory-(3*R*,4*R*)-4-(3-fluoro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (18EN2)

18EN2 was synthesised from **114b** using **General Method XIV**.

Yield: 100mg (0.27mmol) 67%

Appearance: white powder

Melting Point: 144-146 °C

R_f: 0.24 (1:1 *n*-hexane:ethyl acetate)

[α]_D²⁰: -17.6(c 15.1mg/5mL in chloroform) ee (%): 77%

IR ν_{max}(ATR): 3288 cm⁻¹ (OH), 1726 cm⁻¹ (β-lactam C=O)

HRMS: APCI calculated for [M⁺ +H], 378.135181; found 378.134742, error -1.2 ppm

Purity (HPLC): 91%

Ee: 75%

(3*S*,4*S*)-4-(3-Chloro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (19EN1)

19EN1 was synthesised from **117a** using **General Method XIV**.

Yield: 157 mg (0.4 mmol) 71%

Melting Point: 135.9 °C

R_f: 0.34 (1:1; *n*-hexane:ethyl acetate)

IR ν_{max}(ATR): 3321 cm⁻¹ (OH)· 1727 cm⁻¹ (β-lactam C=O)

HRMS: APCI calculated for C₁₉H₂₁ClNO₆ [M +H⁺], 394.106003; found 394.105191, [M⁺ +Na], error -2.1 ppm

Purity (RP-HPLC): 99%

Ee: 91%

(3*R*,4*R*)-4-(3-Chloro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (19EN2)

19EN2 was synthesised from **117b** using **General Method XIV**.

Yield: 53 mg (0.133 mmol) 70%

Melting Point: 135.9 °C

R_f: 0.3 (1:1; *n*-hexane: ethyl acetate)

HRMS: APCI calculated for C₁₉H₂₁ClNO₆ [M+H⁺], 394.105635; found 394.105191, error -1.1 ppm

IR ν_{max}(ATR): 3279 cm⁻¹ (OH)· 1720 cm⁻¹ (β-lactam C=O)

Purity (RP-HPLC): 99%

Ee: 78%

(3*S*,4*S*)-3-Hydroxy-4-(4-methoxy-3-methylphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (20EN1)

20EN1 was obtained from **118a** using **General Method XIV**.

Yield: 99 mg (0.27 mmol) 35%

Melting Point: 128-130 °C

R_f: 0.2 (2:1 *n*- hexane: ethyl acetate), 0.33 (1:1 *n*- hexane: ethyl acetate)

IR ν_{max}(ATR): 3298 cm⁻¹ (OH) , 1725 cm⁻¹ (β-lactam C=O)

HRMS: APCI calculated for $C_{20}H_{24}NO_6$ [$M+H^+$], 374.159814; found 374.159814, error +0.3 ppm

Purity (RP-HPLC): 99%

Ee: 66%

(3R,4R)-3-Hydroxy-4-(4-methoxy-3-methylphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (20EN2)

20EN2 was obtained from **118b** using **General Method XIV**.

Yield: 20 mg (0.054 mmol) 41.2 %

Melting Point: 122-128°C

R_f: 0.2 (2:1 *n*-hexane: ethyl acetate), 0.33 (1:1 *n*-hexane: ethyl acetate),

IR ν_{max} (ATR): 3298 cm^{-1} (OH), 1724 cm^{-1} (β -lactam C=O)

HRMS: APCI calculated for $C_{20}H_{24}NO_6$ [$M+H^+$], 374.159814; found 374.159575, error -0.6 ppm

Ee: 50%

(3S,4S)-4-(3-Bromo-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (21EN1)

21EN1 was synthesised from **120a** using **General Method XIV**.

Yield: 80mg (0.25 mmol) 76%

Melting Point: 63 °C

R_f: 0.25 (1:1; *n*-hexane:ethyl acetate)

IR ν_{max} (ATR): 1747.53 cm^{-1} (β -lactam C=O)

HRMS: APCI calculated for $C_{19}H_{21}BrNO_6$ [$M+H^+$]; 438.054676; found 438.055315; error +1.5 ppm **Ee:** 50%

Ee: 76%

(3R,4R)-4-(3-Bromo-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (21EN2)

21EN2 was synthesised from **120b** using **General Method XIV**.

Yield: 45 mg (0.249 mmol) 78 %

Melting Point: 60-63 °C

R_f: 0.25 (1:1; *n*-hexane:ethyl acetate)

IR ν_{max} (ATR): 1750 cm^{-1} (β -lactam C=O)

HRMS: APCI calculated for $C_{19}H_{21}BrNO_6$ [$M+H^+$]; 438.054676; found 438.054372; error -1.5 ppm

Ee: 96%

(3S,4S)-3-Hydroxy-4-(4-(methylthio)phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (22EN1)

22EN1 was obtained from **119a** using **General Method XIV**.

R_f: 0.25 (1:1; *n*-hexane:ethyl acetate)

IR ν_{max} (ATR): 3300 cm^{-1} (OH), 1721 cm^{-1} (β -lactam C=O)

HRMS: APCI calculated for $C_{19}H_{23}NO_5S$ [$M+H^+$] 376.121320; found 376.121995, error -1.8 ppm

Ee: 84%

(3*R*,4*R*)-3-Hydroxy-4-(4-(methylthio)phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (22EN2)

22EN2 was obtained from **119b** using **General Method XIV**.

Yield: 6 mg (0.02 mmol) 27%

R_f: 0.25 (1:1; *n*-hexane: ethyl acetate)

IR $\nu_{\max}$ (ATR): 3300 cm⁻¹ (OH), 1721 cm⁻¹ (β -lactam C=O).

HRMS: APCI calculated for C₁₉H₂₁NCIO₅S [**M+ Cl⁻**] 410.083445; found 410.084225, error - 1.9 ppm, APCI calculated for C₁₉H₂₂NO₅S [**M+ H⁺**] 376.121320; found 376.121269, error +0.1 ppm, APCI calculated for C₁₀H₂₁NNaO₅S [**M+ Na⁺**] 398.103264; found 398.10254

Ee: 85%

3*S*, 4*R*)-4-(3-Hydroxy-4-methoxyphenyl)-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (24EN1)

24EN1 was synthesised from **124a** using **General Method XIV**.

Yield: 28 mg (0.06 mmol) 43%

R_f: 0.4-0.5 (2:1 *n*-hexane:ethyl acetate)

IR $\nu_{\max}$ (ATR): 3282 cm⁻¹ (OH), 1746 cm⁻¹ (β -lactam C=O)

HRMS: APCI calculated for C₂₅H₂₅NNaO₆ [**M+ Na⁺**], 458.157408; found 458.157727; error - 0.7 ppm

Ee: 96%

(3*R*, 4*S*)-4-(3-Hydroxy-4-methoxyphenyl)-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (24EN2)

24EN2 was synthesised from **124b** using **General Method XIV**.

Yield: 20 mg (0.05 mmol) 38%

R_f: 0.4-0.5 (2:1 *n*-hexane: ethyl acetate)

IR $\nu_{\max}$ (ATR): 3282cm⁻¹ (OH), 1746 cm⁻¹ (β -lactam C=O)

HRMS: APCI calculated for C₂₅H₂₄NNaO₆ [**M -H⁺**], 434.160911; found 434.160898; error - 0.0 ppm

Ee: 59%

4-(3-Hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (27EN1)

(absolute configuration not determined)

27EN1 was synthesised from **125a** using **General Method XIV**.

Yield: 40 mg (0.11 mmols) 58 %

Appearance: yellow powder

R_f: 0.25 (1:1 *n*-hexane: ethyl acetate)

IR $\nu_{\max}$ (ATR) 1731 cm⁻¹ (β -lactam C=O)

HRMS: APCI calculated for C₁₉H₂₂NO₆ [**M+H⁺**]; 360.144164 found 360.144342, error + 0.2 ppm

Ee: 61%

4-(3-Hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (27EN2)

(absolute configuration not determined)

27EN2 was synthesised from **125b** using **General Method XIV**.

Yield: 50 mg (0.134 mmols) 80 %

Appearance: yellow powder

R_f: 0.25 (1:1 *n*-hexane: ethyl acetate)

IR $\nu_{\max}$ (ATR): 1731 cm⁻¹ (β -lactam C=O)

HRMS: APCI calculated for C₁₉H₂₂NO₆ [**M+H**⁺]; 360.144164 found 360.144282, error + 0.3 ppm

Ee: 45%

(3*S*, 4*R*)-4-(3-Hydroxy-4-methoxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one

(30EN1)

30EN1 was synthesised from **127a** using **General Method XIV**.

Yield: 47 mg (0.11 mmol) 63 %

Appearance: yellow powder

Melting Point: 94-96 °C

R_f: 0.3 (1:1 *n*-hexane: ethyl acetate)

HRMS: APCI calculated for C₂₃H₂₄NO₆S [**M+H**⁺]; 442.131885; found 442.1312129, error -0.6 ppm

IR $\nu_{\max}$ (ATR): 1720.29 cm⁻¹ (β -lactam C=O)

Ee: 100%

(3*R*, 4*S*)-4-(3-Hydroxy-4-methoxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (30EN2)

30EN2 was synthesised from **127b** using **General Method XIV**.

Yield: 110 mg (0.25 mmol) 78 %

Appearance: pale yellow powder

Melting Point: 94-96 °C

R_f: 0.28 (1:1 *n*-hexane: ethyl acetate)

IR $\nu_{\max}$ (ATR): 1720.29 cm⁻¹ (β -lactam C=O)

HRMS: APCI calculated for C₂₃H₂₄NO₆S [**M-H**⁺]; 442.131885; found 442, error -0.9 ppm

Ee: 56 %

(3*S*, 4*R*)-4-(3-Hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-one (33EN1)

33EN1 was synthesised from **128a** using **General Method XIV**.

Yield: 30 mg (0.07 mmol) 38 %

Appearance: white powder

R_f: 0.35 (1:1 *n*-hexane: ethyl acetate)

HRMS (APCI): Calculated for C₂₁H₂₂NO₆ [**M-H**⁺]; 384.145261, found 384.145315. Error -0.1 ppm

Ee: 85 %

(3*S*, 4*R*)-3-(4-Fluorophenyl)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (39EN1)

39EN1 was synthesised from **130a** using **General Method XIV**.

Yield: 30 mg (0.07 mmol) 86%

R_f: 0.35 (1:1 *n*-hexane: ethyl acetate)

IR $\nu_{\max}$ (ATR): 1741 cm⁻¹ (β -lactam C=O)

HRMS: APCI calculated for C₂₅H₂₅FNO₆ [M+H⁺], 454.166042; found 454.166042; error + 0.6 ppm

(3*R*, 4*S*)-3-(4-Fluorophenyl)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (39EN2)

39EN2 was synthesised from **139b** using **General Method XIV**.

Yield: 40 mg (0.09 mmol) 81 %

R_f: 0.35 (1:1 *n*-hexane: ethyl acetate)

IR ν_{max}(ATR): 1740 cm⁻¹ (β-lactam C=O)

HRMS: APCI calculated for C₂₅H₂₅FNO₆ [M + H⁺], 454.166042; found 454.165688; error + 0.8 ppm

(3*S*, 4*R*)-4-(3-Hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (41EN1)

41EN1 was synthesised from **131a** using **General Method XIV**.

Yield: 54 mg (0.12 mmol) 85%

Appearance: yellow powder

R_f: 0.35 (1:1 *n*-hexane: ethyl acetate)

IR ν_{max}(ATR): 2937 cm⁻¹ (OH), 1736 cm⁻¹ (C=O)

HRMS: APCI calculated for C₂₆H₂₈NO₇ [M+H⁺] 466.186029; found 466.186117, error + 0.2 ppm

(3*R*, 4*S*)-4-(3-Hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (41EN1)

41EN2 was synthesised from **131b** using **General Method XIV**.

Yield: 47 mg (0.10 mmol) 61%

Appearance: yellow powder

R_f: 0.35 (1:1 *n*-hexane: ethyl acetate)

IR ν_{max}(ATR): 2937 cm⁻¹ (OH), 1736 cm⁻¹ (C=O)

HRMS: APCI calculated for C₂₆H₂₈NO₇ [M+H⁺] 466.186029; found 466.186243, error + 0.2 ppm

General Method XV: Optimised biocatalytic methanolysis of racemic 3-acetoxy β-lactams using *Candida antarctica* lipase B (CAL-B) for isolation of (3*S*, 4*S*) 3-hydroxy and (3*R*, 4*R*) 3-acetoxy β-lactams

3-Acetoxy β-lactam (1 eq) was added to TBME (40 mL) in glass tubes of the Radley 12 plus carousel reaction station. 0.5 mg of CAL-B per mg of 3-acetoxy β-lactam was added to the reaction vessel followed by methanol (3 eq). The reaction was stirred and monitored by ¹H NMR until 35-45% conversion to the 3-hydroxy enantioenriched 3*S*, 4*R* β-lactam (2-6 days). The reaction was quenched *via* filtration of the enzyme and washing the residue with ethyl acetate. The solvent was removed *in vacuo* followed by separation of 3*R*, 4*R* 3-acetoxy β-lactam from the enantioenriched (3*S*, 4*R*) 3-hydroxy β-lactam using flash column chromatography with isocratic elution of 1:1 *n*-hexane: ethyl acetate. Following calculation of enantiomeric excess

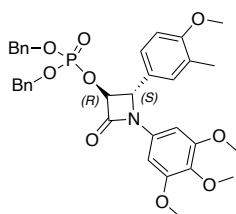
(*ee*) for both analogues using chiral HPLC [with Chrompak-IH-3 (150 × 4.6 mm) column and Chiral- IH-3 guard column, mobile phase 1:1 *n*-hexane: isopropanol], the 3-acetoxy β -lactam was reacted further with 1 mg/mg of CALB, methanol (6 eq) and TBME (40 mL). The reaction was again monitored by ^1H NMR and quenched at 50-60% conversion. Work up and purification was repeated to isolate enantiopure 3*R*,4*R* 3-acetoxy β -lactam.

General Method XVI: Addition of dibenzylphosphite to afford dibenzylphosphate esters

The phenol containing compound (1 eq) was added to a stirring solution of anhydrous MeCN under a nitrogen atmosphere (20 mL) and sonicated for 10 minutes until a homogenous suspension was observed. CCl_4 (3 eq) was added via syringe and septum on an ice/sodium chloride mixture at -20°C and stirred for 15 minutes while maintaining the nitrogenous atmosphere. DMAP (0.2 mmol) and DIPEA (4 mmol) were dissolved in MeCN (2 mL) and injected. Dibenzylphosphite (1.4 eq) was added dropwise to the reaction vessel. The reaction was then warmed to RT and stirred overnight. The reaction was quenched with $\text{KHSO}_4(\text{sat})$ (10 mL). The MeCN/water layer was then extracted with ethyl acetate ($4 \times 15\text{ mL}$). The organic layers were combined and dried with anhydrous Na_2SO_4 . The solvent was removed *in vacuo* and crude product purified using LC and isocratic elution (1:1 *n*-hexane/ethyl acetate).

Dibenzyl ((2*S*,3*R*)-2-(4-methoxy-3-methylphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl) phosphate (140)

20EN1 was isolated using General Method XV and **140** was synthesised from using General Method XVI.



^1H NMR (600 MHz, CDCl_3): δ 7.31 (m, 10H, benzyl), 7.10 (dd, 1H, $J = 8.8\text{ Hz}$, $J = 2.2\text{ Hz}$), 7.05 (apparent s, 1H), 6.78 (d, 1H, $J = 8.8\text{ Hz}$), 6.54 (s, 2H), 5.10-5.06 (m, 5H, H_3 and benzyl $\text{CH}_2 \times 2$), 4.94 (apparent s, 1H, H_4), 3.85 (s, 3H), 3.79 (s, 3H), 3.72 (s, 6H)

^{13}C NMR (100 MHz, CDCl_3): δ 161.1 (d, $J_{\text{C2-P}} = 9\text{ Hz}$), 158.4, 153.5, 135.3, 135.1, 132.9, 128.7, 128.6, 128.1, 127.8, 126.1, 125.3, 110.2, 95.5, 95.4, 84.3 (d, $J_{\text{C3-P}} = 9\text{ Hz}$), 70.0, 63.9, 60.9, 56.1, 55.4

^{31}P NMR (162 MHz, $\text{DMSO}-d_6$): δ - 2.84

***Ee*:** 71%

General Method XVII: Perkin condensation for synthesis of P3 acetate using reflux

A stirring solution of salicaldehyde (40 mmol, 1 eq, 4.84 g, 4.18 mL) and 2-methylpyrazine (40 mmol, 1 eq, 3.76g, 3.65 mL) in acetic anhydride (211 mmol, 5.29 eq, 20 mL) was refluxed at

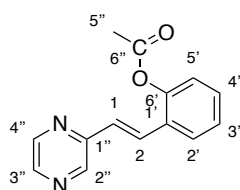
180 °C for 4-5 days and monitored by TLC until visualisation of required product at 365 nm under UV light. A characteristic yellow colouring was seen on day 1 followed by darkening of the reaction vessel thereafter to a brown/black colour. After 4-5 days, the acetic anhydride was reduced *in vacuo*, dried under N₂ gas to afford a brown oil. A mixture of the free phenol and acetate intermediate were observed on TLC and on ¹H NMR. The acetate intermediate was hydrolysed to the free phenol using 1M NaOH (aq.) (1-2 drops) prior to further purification.

General Method XVIII: Perkin condensation for synthesis of P3 acetate using microwave technology

Salicaldehyde (1 eq, 10 mmol, 1.05 mL) and 2-methylpyrazine (1 eq, 10 mmol, 0.92 mL) were added to 5 mL of acetic anhydride in a microwave tube. The reaction vessel was placed in microwave reactor for 5 hours at 160° C on standard power (300 W). After 5 hours the vessel contents were diluted with dH₂O (30 mL) and extracted with ethyl acetate (2 × 40 mL). The extracted organic layer was dried with anhydrous MgSO₄, filtered and solvent reduced *in vacuo*. The crude brown oil was purified using flash column chromatography with a gradient of 1:1 – 2:1, ethyl acetate: *n*-hexane to afford **P3 acetate** as a fine yellow solid.

((*E*)-2-(2-(Pyrazin-2-yl)vinyl)phenyl acetate) (P3 acetate)

P3 acetate was synthesised using **General Method XVII** and **XVIII**.



Yield: Not calculated for Perkin condensation using reflux. 17% using microwave technology

Appearance: fine yellow powder.

Melting Point: 131-132 °C

R_f: 0.55 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (600 MHz, DMSO-*d*₆): δ 8.96 (d, 1H, *J* = 1.1 Hz, H_{2''}), 8.66 (apparent triplet, 1H, *J* = 1.5 Hz, H_{3''}), 8.54 (d, 1H, *J* = 2.4 Hz, H_{4''}), 7.93 (dd, 1H, *J* = 7.22, *J* = 1.5 Hz, H_{2'}), 7.75 (d, 1H, *J* = 16 Hz, CH=CH, H₂), 7.43 (d, 1H, *J* = 16 Hz, CH=CH, H_{1''}), 7.42 (dd, 1H, *J* = 1 Hz, *J* = 9 Hz, H_{3'}), 7.36 (t, 1H, *J* = 7.6 Hz, H_{4'}), 7.2 (dd, 1H, *J* = 8.4 Hz, *J* = 1.3 Hz, H_{5'}), 2.39 (s, 3H, COOCH₃, H_{5''})

¹³C NMR (100 MHz, DMSO-*d*₆): δ 169.1 (C_{6''}), 150.8 (C_{1''}), 149.9 (C_{6'}), 145.1 (C_{3''}), 144.6 (C_{2''}), 144.0 (C_{4''}), 130.4 (C_{3'}), 129.0 (C_{2'}), 128.0 (C_{2'}), 127.4 (C₂), 127.2 (C₁), 126.9 (C_{4'}), 123.8 (C_{5'}), 23.9 (C_{5''})

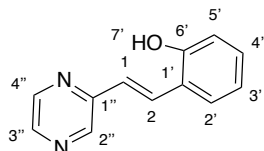
HRMS: APCI calculated for C₁₄H₁₃N₂O₂ [**M+H**⁺]: 241.097154, found 241.06916, error + 0.2 ppm

General Method XIX: Hydrolysis of Perkin condensation product P3 acetate to free phenolic P3.

P3 acetate was added to a stirring solution of 1:1 ethyl acetate/ethanol (40 mL). 1M NaOH (1-2 mL) was added to this suspension which was left to stir vigorously until completion as indicated by TLC (10-20 minutes). The solvent was then removed *in vacuo*. The crude product was purified using dry loading in methanol over silica gel using gradient elution to remove residual salicaldehyde (9:1 to 1:2 hexane:ethyl acetate).

(E)-2-(2-(Pyrazin-2-yl)vinyl)phenol (P3)

P3 was synthesised from **P3 acetate** using **General Method XIX**.



Yield: 1.5-2g (< 25%)

Melting Point: 206-209 °C

Appearance: Bright yellow crystalline solid

R_f: 0.5 1:1 *n*-hexane: ethyl acetate

¹H NMR (600 MHz, DMSO-*d*₆): δ 9.97 (bs, 1H, H_{7'}), 8.70 (s, 1H, H_{2''}), 8.51 (apparent s, 1H, H_{4''}), 8.44 (d, 1H, *J* = 2.4 Hz, H_{3''}), 8.09 (d, 1H, *J* = 16.2 Hz, H₁), 7.62 (1H, *J* = 8 Hz, H_{5'}), 7.34 (d, 1H, *J* = 16.2 Hz, H₂), 7.15 (t, 1H, *J* = 7.2 Hz, H_{3'}), 6.89 (d, 1H, *J* = 6.5 Hz, H_{2'}), 6.83 (1H, *J* = 6.5 Hz, H_{3'})

¹³C NMR (100 MHz, DMSO-*d*₆): δ 156.4 (C_{6'}), 151.7 (C_{1''}), 144.9 (C_{4''}), 144.2 (C_{2''}), 143.17 (C_{3''}), 130.5 (C_{3'}), 130.4 (C₁), 128.0 (C_{5'}), 124.0 (C₂), 123.2 (C_{1'}), 119.9 (C_{3'}), 116.5 (C_{2'})

HRMS: APCI Calculated for C₁₂H₉N₂O [**M-H**⁺]: 197.072405, found 197.0236, error + 1.9 ppm

((E)-Dibenzyl (3-(2-(pyrazin-2-yl)vinyl)phenyl) phosphate) (P3DBP)

P3DBP was synthesised from **P3** and dibenzyl phosphite using **General Method XVI**.

R_f: 0.45 (1:1; *n*-hexane: ethyl acetate)

Appearance: Yellow oil

Yield: 60%

¹H NMR (600 MHz, DMSO-*d*₆): δ 8.70 (s, 1H), 8.62 (s, 1H), 8.53 (d, 1H, *J* = 2.2 Hz), 8.04 (d, 1H, *J* = 16 Hz), 7.91 (d, 1H, *J* = 6.7 Hz), 7.43 (d, 1H, *J* = 16 Hz), 7.33 (complex m, 11H), 5.20 (s, 2H), 5.19 (s, 2H).

¹³C NMR (100 MHz, DMSO-*d*₆): δ 150.2, 148.1, 148.0, 144.6, 143.5, 135.5, 135.5, 130.1, 128.5, 128.4, 127.9, 127.7, 127.6, 127.1, 127.0, 126.4, 125.7, 129.7, 69.5, 69.4

³¹P NMR (162 MHz, DMSO-*d*₆): δ -6.1ppm

HRMS: APCI calculated for C₂₆H₂₄N₂O₄P [**M+H**⁺], 459.146881; found 459.146820, error + 0.1 ppm

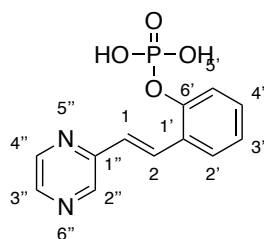
ESI calculated for C₂₆H₂₄N₂O₄PNa [**M+Na**⁺], 481.128765; found 481.128652, error -0.2 ppm

General Method XX: Debenzylation of P3 dibenzyl phosphate to P3P using boron tribromide

P3DBP (200 mg, 0.5 mmol, 1 eq) was weighed and added to a stirring solution of anhydrous toluene (20 mL), under N₂ and on ice water/methanol trough at -10 °C. Boron tribromide 1M in anhydrous toluene (0.5 mmol, 1 eq, 0.5 mL) was added dropwise to the stirring solution while maintaining a temperature of -10 °C. The reaction was warmed to room temperature before bringing the temperature to 80 °C. On completion of the debenzylation after two hours reflux, a yellow precipitate was observed. The reaction vessel was cooled once again to room temperature. An excess of methanol (10mL) was then added. The solvent was then removed *in vacuo* and left under vacuum conditions at 60 °C to allow for evaporation of the excess boron tribromide. The crude solids were purified using Sephadex gel filtration (Sephadex® G-10 Medium) to afford the pure phosphoric acid derivative as a solid yellow powder.

(E)-2-(2-(Pyrazin-2-yl)vinyl)phenyl dihydrogen phosphate (P3P)

P3 phosphoric acid (**P3P**) was synthesised from **P3DBP** using **General Method XX**.



Yield: 55mg (0.198 mmol) 40%

Appearance: bright yellow powder.

R_f: 0.05 (4:1 ethyl acetate: methanol)

¹H NMR (600 MHz, DMSO-*d*₆): δ 8.75 (apparent singlet, 1H, H_{4''}), 8.64 (apparent singlet, 1H, H_{3''}), 8.50 (d, 1H, *J*_{2''-3''} = 2.2 Hz, H_{2''}), 8.04 (d, 1H, *J*₂₋₁ = 16.4 Hz, H₂), 7.83 (d, 1H, *J*_{2'-3'} = 8.2 Hz, H_{2'}), 7.4 (d, 1H, *J*₁₋₂ = 15.98 Hz, H₂), 7.44 (d, 1H, *J*_{5'-4'} = 4.6 Hz, H_{5'}), 7.31 (t, 1H, *J*_{4'-3'-5'} = 7.3 Hz, H_{4'}), 7.17 (t, 1H, *J*_{3'-4'-2'} = 7.3 Hz, H_{3'})

¹³C NMR (100 MHz, DMSO-*d*₆): δ 151.3 (C_{1''}), 150.7 (d, *J*_{P-C6'} = 8.1 Hz), 145.0 (C_{3''}), 144.4 (C_{4''}), 143.6 (C_{2''}), 130.7 (C_{4'}), 129.2 (C₂), 127.7 (d, *J*_{P-C1'} = 7.6 Hz, C_{1'}), 127.1 (C_{2'}), 125.6 (C₁), 124.2 (C_{3'}), 121.2 (C_{5'})

³¹P NMR (162 MHz, DMSO-*d*₆): δ -6.1

¹⁵N NMR (41 MHz, DMSO-*d*₆): δ 341.4 (N_{5''}), 321.0 (N_{6''})

HRMS: APCI Calculated for C₁₂H₁₂N₂O₄P [**M+H**⁺]: 279.05290, found 279.052943, error + 0.1 ppm

General Method XXI: Aminocatalytic synthesis of P3 imines using pyrrolidine

2-Nitrobenzaldehyde (1 mmol, 1 eq, 151 mg) and aminopyrazine (1 mmol, 1 eq, 95 mg) was added to 10 mL of ethanol under a nitrogen atmosphere containing 20 mol % pyrrolidine catalyst (0.2 eq, 0.2 mmol, 1 μL). The reaction was refluxed at 90° C for 7 hours. Completion was not achieved. The solvent was reduced *in vacuo* and imine used prior to further purification

General Method XXII: Synthesis of P3 Imines using TiCl₄

TiCl₄ (2.5 mmol, 0.5 eq, 2.5 mL) was added slowly to anhydrous toluene (40 mL) under nitrogenous conditions at 0 °C prior to addition of 2-nitrobenzaldehyde (5 mmol, 1 eq, 755 mg). After stirring for 10 minutes, 2-aminopyrazine (5 mmol, 1 eq, 475 mg) was added. Tri-*n*-butylamine (15 mmol, 3 eq, 3.57 mL) was added dropwise while ensuring to keep the temperature at 0 °C. The reaction was allowed to stir for 30 minutes at 0 °C prior to warming to room temperature and stirring overnight. The reaction was quenched with sodium bicarbonate (5 mL). The organic layer was diluted with ethyl acetate (20 mL), separated from aqueous layer and dried with anhydrous Na₂SO₄ prior to removal of the solvent *in vacuo*. The crude imine was used without further purification.

General Method XXIII: Imine Synthesis using Dean Stark apparatus, ethanol and toluene

Aminopyrazine (5 mmol, 1 eq, 475 mg) was dissolved in ethanol (5 mL) prior to addition to anhydrous toluene (30 mL) under reflux conditions using the Dean Stark apparatus at 100 °C. The amino pyrazine was allowed to dissolve in the reaction vessel prior to addition of the aldehyde for 10-15 minutes. 2-Nitrobenzaldehyde (5 mmol, 1 eq, 755 mg) was subsequently added and the mixture was refluxed for 48-72 hours until formation of imine was evident on TLC. The solvent was reduced *in vacuo* and imine used without further purification.

(*E*)-1-(2-Nitrophenyl)-*N*-(pyrazin-2-yl)methanimine (149**)**

149 was synthesised using **General Method XXI, XXII and XXIII**.

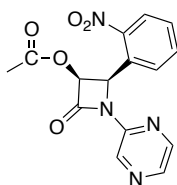
R_f: 0.64 (1:1 ethyl acetate: *n*-hexane)

Yield: Not calculated. *Imine used without further purification*.

HRMS: (APCI): Calculated for C₁₁H₉N₄O₂, [M+H⁺] 229.072002, found 229.071942, error + 0.3 ppm.

2-(2-Nitrophenyl)-4-oxo-1-(pyrazin-2-yl)azetidin-3-yl acetate (151 cis**)**

141 was synthesised from **149** using **General Method IV** in 9:1 *cis:trans* ratio



R_f: 0.47 (3:2 ethyl acetate: *n*-hexane)

Appearance: yellow powder

Yield for 151: (Imine **149** synthesised using Dean Stark, toluene and ethanol as co-solvent): 210 mg (0.64 mmol) 24%

Yield for 151: (Imine **149** synthesised using aminocatalysis): 160 mg (0.5 mmol) 25%

Yield for 151: (Imine **149** synthesised using TiCl₄): 20 mg (0.06 mmol) 1.21%

¹H NMR (600 MHz, CDCl₃): δ 8.42 (d, 1H, *J* = 2.9 Hz), 8.26 (dd, 1H, *J* = 7.2 Hz, 2.2 Hz), 8.2 (apparent multiplet, 1H), 7.65 (td, 1H, *J* = 2.2 Hz, *J* = 6.7 Hz), 7.58 (td, 1H, *J* = 2.2 Hz, *J* = 6.7

Hz), 7.4 (d, 1H, $J = 7.4$ Hz), 6.46 d, 1H, $J = 6.2$ Hz), 6.28 (d, 1H, $J = 6.2$ Hz), 1.89 (s, 3H, OCOCH₃)

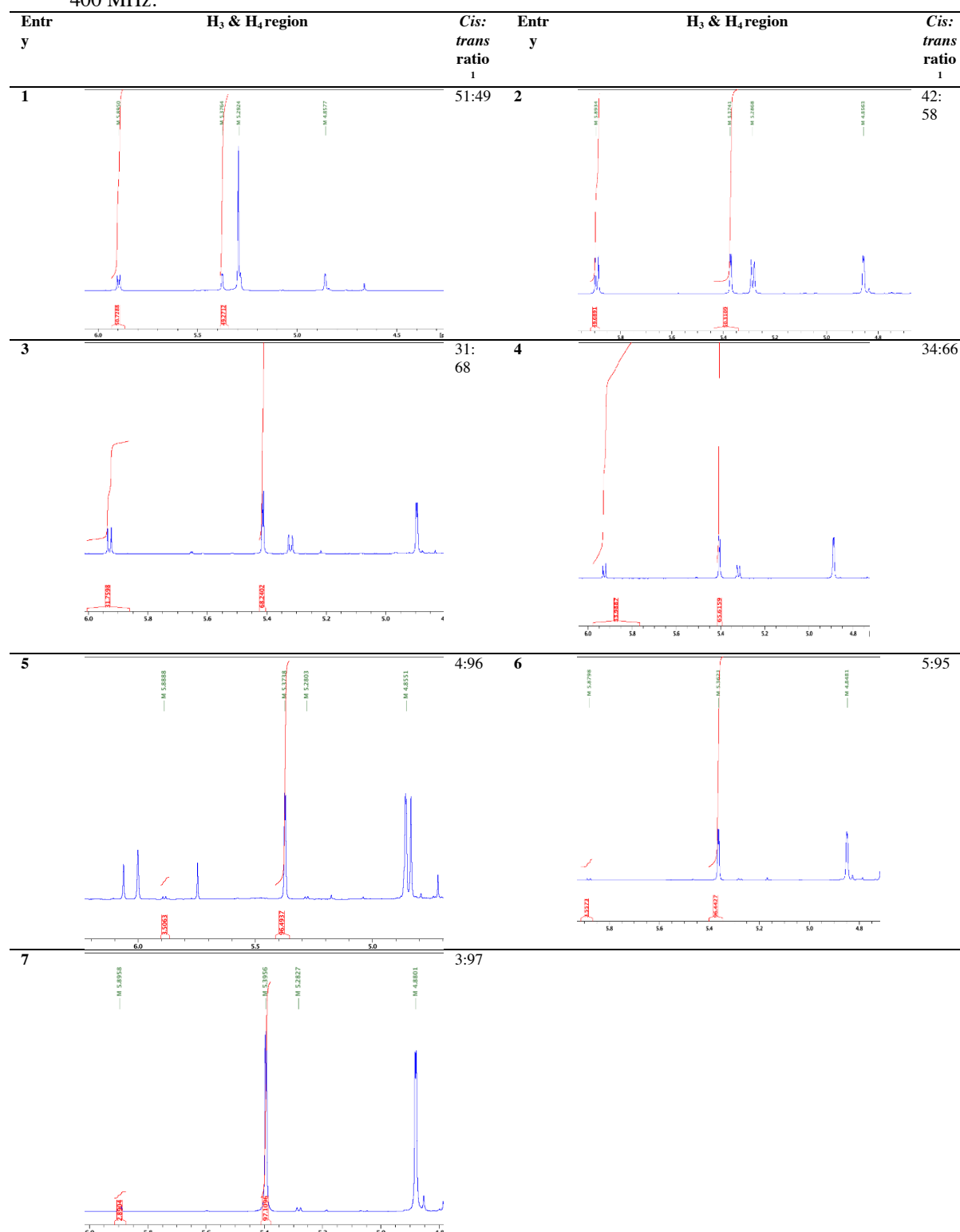
¹³C NMR (100 MHz, CDCl₃): δ 162.4 (β -lactam C=O), 147.6, 145.7, 142.4, 141.2, 137.0, 134.0, 129.3, 128.8, 125.5, 76.1, 58.0, 31.1, 19.8

HRMS: APCI calculated for C₁₅H₁₃N₄O₅ [**M+H**⁺] 329.088046; found 329.087494, error + 1.7 ppm

Appendices

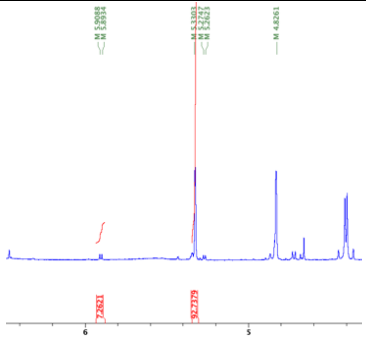
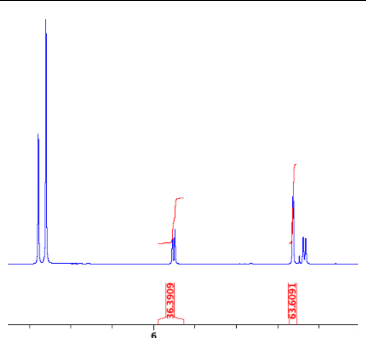
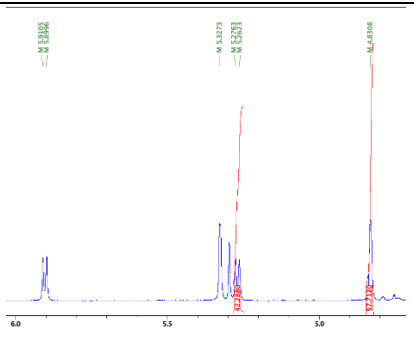
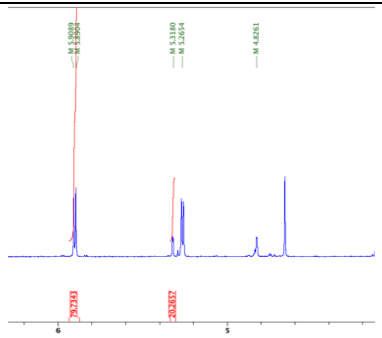
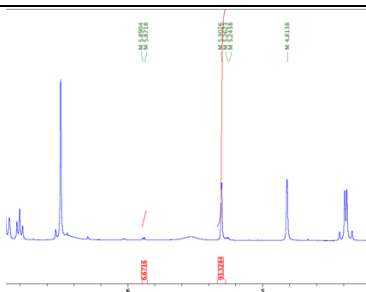
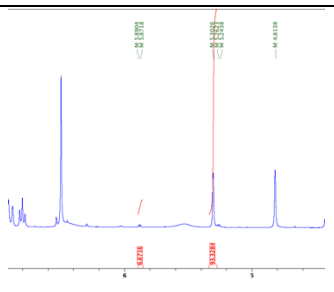
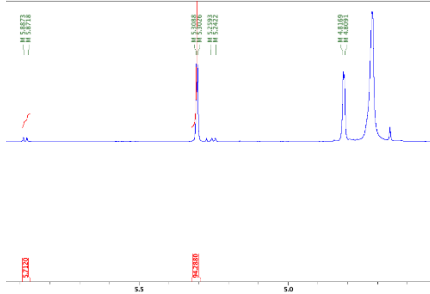
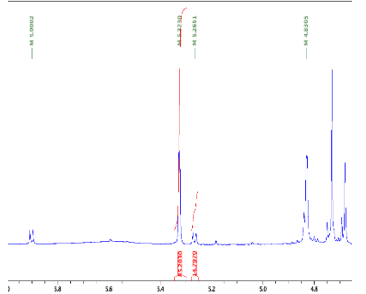
Appendix 1: Additional material related to Chapter 2: Synthesis and chemistry of trans β -lactam racemates

^1H NMR of β -lactam H_3 and H_4 region (δ 4.1- 6.0 ppm) for crude 3-acetoxy- β -lactam **17a** in CDCl_3 at 400 MHz.



¹ Ratios calculated *via* integration of H_3 and H_4 and normalization to 100% for *cis* and *trans* H_3 signals. J = 1.8-2 Hz for *trans* isomer J = 4-5 Hz for *cis* isomer. *Trans* H_3 & H_4 signals appear at δ 4.86 and 5.37 ppm respectively. *Cis* H_3 & H_4 signals appear at δ 5.29 and 5.90 respectively.

¹H NMR of β-lactam H₃ and H₄ region (δ 4.1- 6.0 ppm) for β-lactam **18a** in CDCl₃ at 400 MHz.

Entry	H ₃ & H ₄ region	<i>Cis: trans</i> ratio ¹	Entry	H ₃ & H ₄ region	<i>Cis: trans</i> ratio ¹
1		7:93	2		36:64
3		42:58	4		80:20
5		6:94	6		6:94
7		6:94	8		15:85

¹ Ratios calculated *via* integration of H₃ and H₄ and normalisation to 100% for *cis* and *trans* H₃ signals. *J* = 1.8-2 Hz for *trans* isomer *J* = 4-5 Hz for *cis* isomer. *Trans* H₃ & H₄ signals appear at δ 4.83 and 5.32 ppm respectively. *Cis* H₃ & H₄ signals appear at δ 5.26 and 5.90 respectively.

Stereochemical outcomes for Staudinger reactions 1-3 using optimised Staudinger conditions for isolation of 3-acetoxy- **63a**

Entry	1	2	3
<i>Cis</i> :	22:78	17:83	16:84
<i>Trans</i>			
Yield ¹			

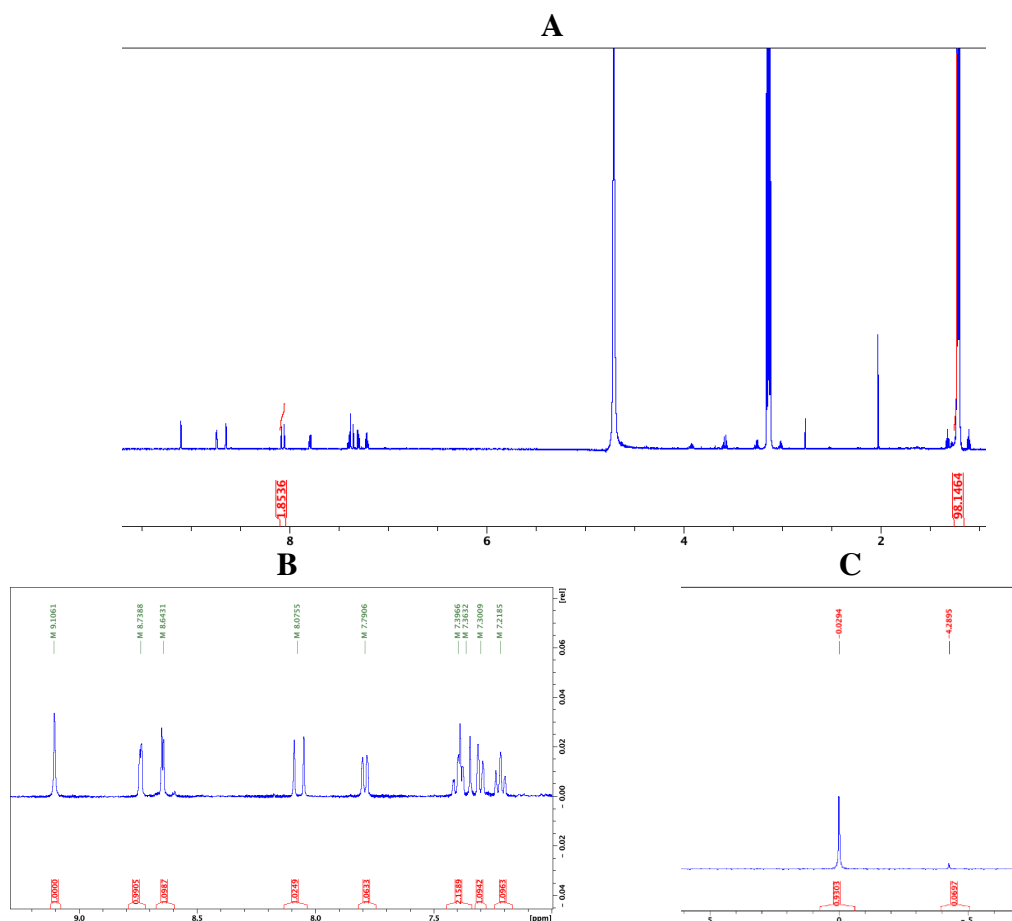
¹Calculated *via* integration of H₃ at δ 5.32 (*trans*) and δ 5.37 ppm (*cis*) followed by normalization to 100%. *J* values for *trans* isomer = 1.8 Hz and *cis* isomer = 3 Hz.

Reflux versus microwave (MAOS) *cis:trans* yields for **77** in crude Reformatsky products calculated using ¹H NMR spectrum in CDCl₃ at 400 MHz.

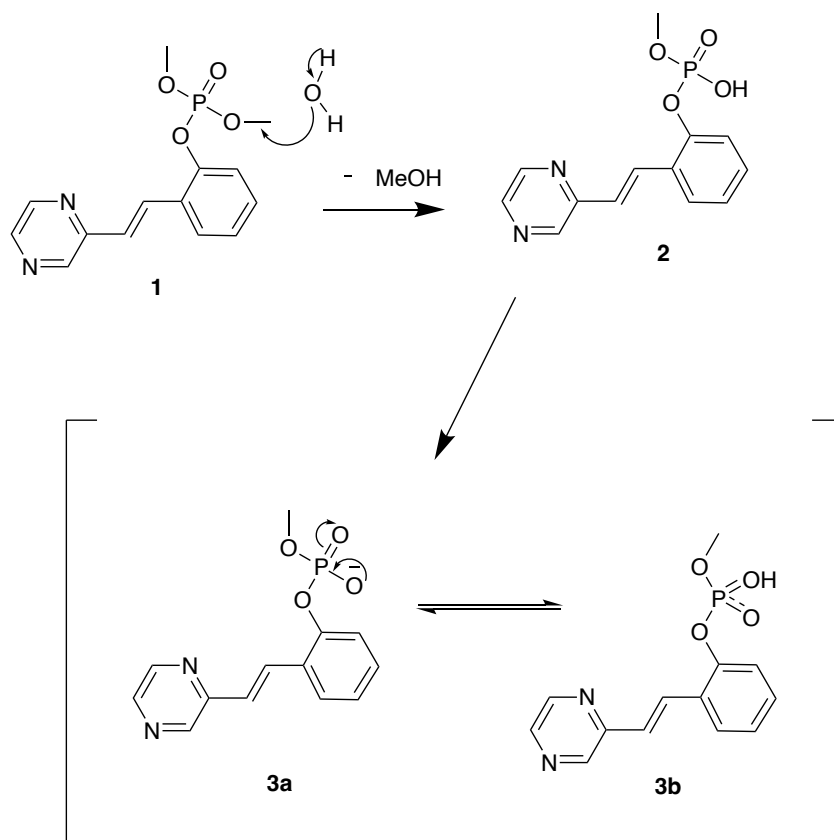
	Reflux		MAOS
<i>Cis: trans</i> ratio ¹	H ₄ region on ¹ H NMR in CDCl ₃ at 400 MHz ²	<i>Cis: trans</i> ratio ¹	H ₄ region on ¹ H NMR in CDCl ₃ at 400 MHz ²
1 76:23		1 58:42	

¹Ratios are calculated by integration of H₄ doublets followed by normalisation to 100%. ²Coupling constants are *J* = 5.5 Hz for *cis* isomer and *J* = 2.2 Hz for *trans* isomer.

Appendix 2: Additional material related to Chapter 8: Physiochemical analysis, prodrug synthesis and structure optimisation of pyrazinib



NMR spectroscopy data of **P3P** isolated using POCl_3/TEA **A:** ^1H NMR in $\text{DMSO}-d_6$ at 600 MHz integrating TEA: **P3P** and normaliaing to 100% to afford a ratio of 98:2. **B:** Enlargement of **P3P** aromatic region from δ 9.5 -7 ppm at 600 MHz in $\text{DMSO}-d_6$. **C:** ^{31}P for **P3P** at 162 MHz in $\text{DMSO}-d_6$.



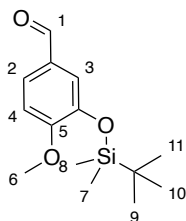
Spontaneous hydrolysis of **P3DMP** (**1**) mediated by atmospheric water to the monomethyl phosphate derivative.³⁴² Ionisation of **2** yields phosphate anion **3a** in equilibrium with resonance structure **3b** (The electrophilic phosphate of the dimethyl derivative (**1**) undergoes nucleophilic attack by water to yield a methanol by-product and a monomethylated phosphate ester.³⁴² The monomethylated ester is acidic in nature and has potential for ionisation to **3a**, the monomethylated phosphate anion in equilibrium with **3b** the resonance delocalised phosphate anion. Resonance delocalisation of the O⁻ negative charge reduces the electrophilicity of the phosphate atom, thus preventing a second demethylation mediated by water as a weak nucleophile to the free phosphoric acid.

Solubility data for **P3** and phosphate prodrug **P3P** for three independent studies with average solubility reported as $\mu\text{g/mL} \pm \text{SEM}$

Solubility data for P3		Solubility data for P3P	
Replicate 1	0.046 $\mu\text{g/mL}$	Replicate 1	324.077
Replicate 2	0.067 $\mu\text{g/mL}$	Replicate 2	278.651
Replicate 3	0.040 $\mu\text{g/mL}$	Replicate 3	326.879
Average solubility $\pm$ SEM	0.051 $\pm$ 0.014 $\mu\text{g/mL}$	Average solubility $\pm$ SEM	309.869 $\pm$ 27.071 $\mu\text{g/mL}$

3-((*Tert*-butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (42) ²⁸⁶

42 was synthesised from 3-hydroxy-4-methoxybenzaldehyde using **General Method I, Route B**.



Yield: 2.6g (8.8 mmol) 88%

Appearance: Yellow oil

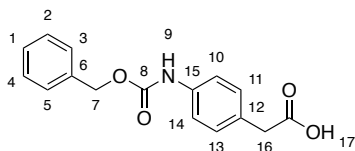
R_f: 0.78 (1:1; *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 9.77 (s, 1H, H₁), 7.43 (dd, *J*= 8.5 Hz, *J*= 2 Hz, 1H, H₂), 7.33 (d, 1H, *J*= 2.1 Hz, H₄), 6.92 (d, 1H, *J*= 8.31 Hz, H₃), 3.86 (s, 3H, OCH₃, H₆), 0.973 (s, 9H, C(CH₃)₃, H_{9,10,11}), 0.86 (s, 6H, SiCH₃, H_{7&8})

¹³C NMR (100 MHz, CDCl₃): δ 190.7 (C₁), 156.6 (C₄), 145.6 (C₅), 130.1, 126.5, 120.0, 111.2, 55.5, 25.6, 25.5, 18.4, 18.0

(4-Benzyloxycarbonylaminophenyl)acetic acid ⁴⁹⁵

(4-Benzyloxycarbonylaminophenyl)acetic acid was synthesised using **General Method II**.



Yield: 1.03g (3.7 mmol) 75%

Appearance: off white/yellow amorphous powder

R_f: 0.42 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, DMSO-*d*₆): δ 9.74 (bs, 1H, H₉), 7.43 (m, 5H, H₁₋₅), 7.32 (d, 2H, H_{10&14}), 7.15 (d, 2H, H_{11&13}), 5.14 (s, 2H, H₇), 3.46 (s, 2H, H₁₆)

¹³C NMR (100 MHz, DMSO-*d*₆): δ 173.6, 153.9, 143.0, 138.0, 137.1, 130.2, 129.5, 128.9, 128.6, 128.5, 127.1, 126.9, 118.6, 63.4, 66.2

Numbering nomenclature for imines

Imine code	R ₁	R ₂
44	H	OCH ₃
45	F	OCH ₃
46	OH	OCH ₃
47	OTBDMS	OCH ₃
48	Cl	OCH ₃
49	CH ₃	OCH ₃
50	H	SCH ₃
51	H	F
52	H	NO ₂
53	Br	OCH ₃
54	NO ₂	OCH ₃
55	H	OCH ₂ CH ₃
56	H	SCH ₂ CH ₃

(*E*)-1-(4-Methoxyphenyl)-*N*-(3,4,5-trimethoxyphenyl)methanimine (**44**)³⁷⁸

44 was synthesised from 4-methoxybenzaldehyde using **General Method III, Method A**.

Yield: 88%

Appearance: bright yellow crystalline solid

Melting Point: 119.1-119.5 °C

R_f: 0.6. (1:1; *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 8.4 (s, 1H, H₇), 7.8 (d, 2H, *J*=8.7 Hz, H_{3&4}), 6.9 (d, 2H, *J*=8.7 Hz, H_{5&6}), 6.45 (s, 2H, H_{1&2}), 3.83 (s, 6H, H_{8&10}), 3.81 (s, 3H, H₁₁), 3.71 (s, 3H, H₉)

¹³C NMR (100 MHz, CDCl₃): δ 159.3, 153.6, 139.6, 114.1, 97.7, 60.9, 56.1, 55.3

(*E*)-1-(3-Fluoro-4-methoxyphenyl)-*N*-(3,4,5-trimethoxyphenyl)methanimine (**45**)⁴⁰⁰

45 was synthesised from 3-fluoro-4-methoxybenzaldehyde using **General Method III, Method A**.

Yield: 90%

Appearance: bright yellow crystalline solid

R_f: 0.40 (1:1; *n*-hexane: ethyl acetate)

Melting Point: 107.1-108.6 °C

¹H NMR (400 MHz, CDCl₃): δ 8.35 (1H, s, H₇), 7.98 (d, 1H, *J*=2 Hz, H₅), 7.75 (1H, apparent doublet, *J*=8.2 Hz, H₄), 6.99 (d, 1H, *J*=9 Hz, H₃), 6.48 (s, 2H, H₁ & H₂), 3.96 (s, 3H, H₁₁), 3.88 (s, 6H, H_{8&10}), 3.85 (s, 3H, H₉)

¹³C NMR (100MHz, CDCl₃): δ 157.6, 153.5, 136.3, 111.8, 101.3 98.2, 70.0, 61.0, 56.4, 56.

¹⁹F NMR (376 MHz, CDCl₃): δ -134.3

(*E*)-2-Methoxy-5-(((3,4,5-trimethoxyphenyl)imino)methyl)phenol (**46**)³⁷⁸

46 was synthesised from 3-hydroxy-4-methoxybenzaldehyde using **General Method III, Method A**.

Yield: 60 %

Appearance: Bright yellow crystalline solid

Melting Point: 165 °C

R_f: 0.5 (1:1; *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 8.33 (s, 1H, H₇), 8.00 (bs, OH, H₁₀), 7.50 (d, 1H, *J*=1.8 Hz, H₃), 7.34 (dd, 1H, *J*=8.5 Hz, *J*=1.8 Hz, H₄), 6.92 (d, 1H, *J*=8.5 Hz, H₅), 6.45 (s, 2H, H_{1&2}), 3.95 (s, 3H, H₇), 3.88 (s, 6H, H_{8&11}), 3.84 (s, 3H, H₉)

¹³C NMR (100 MHz, CDCl₃): δ 159.2, 153.5, 149.3, 148.1, 147.4, 146.0, 129.0, 122.3, 113.8, 110.3, 98.2, 61.0, 56.1

(*E*)-1-(3-((*Tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-*N*-(3,4,5 trimethoxyphenyl) methanimine (47**)³⁷⁸**

47 was synthesised from **42** using **General Method III, Method A**.

Yield: 77%

Appearance: yellow/brown powder

R_f: 0.7 (1:1; *n*-hexane: ethyl acetate)

Melting Point: 79-82 °C

¹H NMR (400 MHz, CDCl₃): δ 8.34 (s, 1H, H₇), 7.43 (s, 1H, H₄), 7.36 (d, 1H, *J*= 8 Hz, H₅), 6.9 (d, 1H, *J*= 8.6 Hz, H₃), 6.45 (s, 2H, H_{1&2}), 3.89 (s, 6H, H_{8&10}), 3.87 (s, 3H, H₉), 3.85 (s, 3H, H₁₁), 1.01, (s, 9H, C(CH₃)₃), 0.18 (s, 6H, C(CH₂)₂)

¹³C NMR (100 MHz, CDCl₃): δ 159.3, 154.0, 153.5, 153.2, 148.3, 145.3, 129.4, 123.8, 120.7, 111.4, 98.1, 61.0, 56.1, 55.5, 25.7, 18.4

(*E*)-1-(3-Chloro-4-methoxyphenyl)-*N*-(3,4,5-trimethoxyphenyl)methanimine (48**)⁴⁰⁰**

48 was synthesised from 3-chloro-4-methoxybenzaldehyde using **General Method III, Method B**.

Yield: 98%

Appearance: Bright yellow crystalline solid

Melting Point: 99.3-100.7 °C

R_f: 0.66 (1:1; *n*-hexane: ethyl acetate); 0.51 (2:1; *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 8.36 (1H, s, H₇), 7.71 (dd, 1H, *J*=12 Hz, *J*=1.8 Hz, H₃), 7.54 (d, 1H, *J*=8.6 Hz, H₅), 7.03 (apparent triplet, 1H, *J*=8.4 Hz, H₄), 6.47 (s, 2H, H_{1&2}), 3.95 (s, 3H, H₁₁), 3.89 (s, 6H, H_{8&10}), 3.86 (s, 3H, H₉)

¹³C NMR (100 MHz, CDCl₃): δ 157.5, 157.4, 153.6, 147.6, 136.4, 130.1, 129.8, 128.9, 111.7, 98.2, 61.0, 56.3, 56.1

(*E*)-1-(4-Methoxy-3-methylphenyl)-*N*-(3,4,5-trimethoxyphenyl)methanimine (49**)⁴⁰⁰**

49 was synthesised from 3-methyl-4-methoxybenzaldehyde and 3,4,5-trimethoxyaniline by **General Method III Method B**.

Yield: 72%

Appearance: Yellow crystals

Melting Point: 140-141 °C

R_f: 0.76 (1:1; *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 8.40 (s, 1H, H₇), 7.68 (d, 1H, *J*=8 Hz, H₅), 6.91 (d, 1H, *J*= 8 Hz, H₃), 6.51 (s, 2H, H_{1&2}), 5.95 (s, 1H), 3.91 (s, 6H, H_{8&10}), 3.88 (s, 3H, H₁₁), 3.82 (s, 3H, H₉), 2.30 (s, 3H, CH₃)
¹³C NMR (100 MHz, CDCl₃): δ 160.6, 159.6, 142.9, 135.6, 130.43, 129.0, 127.4, 125.2, 109.7, 98.2, 92.71, 61.1, 61.2, 56.1, 56.0, 55.6, 24.0

(*E*)-1-(4-(Methylthio)phenyl)-*N*-(3,4,5-trimethoxyphenyl)methanimine (50) ³⁷⁸

50 was synthesised from 4-(methylmercapto)benzaldehyde using **General Method III, Method B**.

Yield: 95%

Appearance: Pale yellow solid.

Melting Point: 119-121 °C

R_f: 0.55 (1:1; *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 8.43 (s, 1H, H₇), 7.82 (d, 2H, *J*=9 Hz, H_{3&4}), 7.3 (d, 2H, *J*= 7.7 Hz, H_{5&6}), 6.51 (s, 2H, H_{1&2}), 3.92 (s, 6H, H_{8&10}), 3.87 (s, 3H), 2.55 (s, 3H, S(CH₃))

¹³C NMR (100 MHz, CDCl₃): δ 158.9, 153.6, 147.9, 143.4, 142.9, 136.4, 132.62, 129.94, 129.0, 125.7, 98.3, 61.1, 56.2, 56.1, 45.0

IR ν_{max}(ATR): 1583 cm⁻¹ (C=N)

(*E*)-1-(4-Fluorophenyl)-*N*-(3,4,5-trimethoxyphenyl)methanimine (51)

51 was synthesised from 4-fluorobenzaldehyde using **General Method III, Method B**.

Yield: 95%

Appearance: Pale yellow powdery solid

R_f: 0.7 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 8.45 (s, 1H, H₇), 7.92 (apparent multiplet, 2H, H_{5&6}), 7.18 (apparent triplet, 2H, H_{3&4}), 6.50 (s, 2H, H_{1&2}), 3.93 (s, 6H, H_{8&10}), 3.89 (s, 3H, H₉)

¹³C NMR (100 MHz, CDCl₃): δ 158.2, 153.5, 147.7, 130.9, 130.7, 116.1, 115.9, 98.1, 61.1, 57.9, 56.1

¹⁹F NMR (376 MHz, CDCl₃): δ -107.9

(*E*)-1-(4-Nitrophenyl)-*N*-(3,4,5-trimethoxyphenyl)methanimine (52)

52 was synthesised from 4-nitrobenzaldehyde and 3,4,5-trimethoxyaniline by **General Method III, Method B**.

Yield: >90% **Appearance:** pale yellow. solid

R_f: 0.51 (1:1; *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 8.59 (s, 1H, H₇), 8.36 (d, 2H, *J*= 9.1 Hz, H_{5&6}), 8.1 (d, 2H, *J*= 9.1 Hz, H_{3&4}), 6.58 (s, 2H, H_{1&2}), 3.95 (s, 6H, H_{8&10}), 3.91 (s, 3H, H₉)

¹³C NMR (100 MHz, CDCl₃): δ 156.5, 149.0, 146.7, 141.4, 129.3, 124.1, 98.4, 61.1, 56.2

(*E*)-1-(3-Bromo-4-methoxyphenyl)-*N*-(3,4,5-trimethoxyphenyl)methanimine (53) ³⁷⁸

53 was synthesised from 3-bromo-4-methoxybenzaldehyde using **General Method III, Method B**.

Yield: > 90%

Appearance: Bright yellow solid

R_f: 0.65 (1:1; *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 8.36 (s, 1H, H₇), 8.30 (s, 1H, H₄), 8.04 (d, 2H, *J* = 10.8 Hz, H₅), 7.12 (d, 1H *J* = 9 Hz, H₃), 6.44 (s, 2H, H_{1&2}), 3.97 (s, 3H, H₉), 3.84 (s, 6H, H_{8&10}), 3.80 (s, 3H, H₆)

¹³C NMR (100 MHz, CDCl₃): δ 151.4, 150.1, 148.9, 142.2, 135.1, 132.1, 128.9, 124.2, 108.9, 93.5, 56.2, 53.7, 52.1, 51.4

(*E*)-1-(4-Methoxy-3-nitrophenyl)-*N*-(3,4,5-trimethoxyphenyl)methanimine (54)

54 was synthesised from 4-methoxy-3-nitrobenzaldehyde using **General Method III, Method B**.

Yield: > 90%

Appearance: Yellow solid

Melting Point: 145-149 °C

R_f: 0.55 (1:1; *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 8.36 (s, 1H, H₇), 8.30 (s, 1H, H₄), 8.04 (d, 2H, *J* = 10.8 Hz, H₅), 7.12 (d, 1H *J* = 9 Hz, H₃), 6.44 (s, 2H, H_{1&2}), 3.97 (s, 3H, H₉), 3.84 (s, 6H, H_{8&10}), 3.80 (s, 3H, H₆)

¹³C NMR (100 MHz, CDCl₃): δ 151.4, 150.1, 148.9, 142.2, 135.1, 132.1, 128.9, 124.2, 108.9, 93.5, 56.2, 53.7, 52.1, 51.4

(*E*)-1-(4-Ethylphenyl)-*N*-(3,4,5-trimethoxyphenyl)methanimine (55)

55 was synthesised from 4-methoxy-3-nitrobenzaldehyde using **General Method III, Method A**.

Yield: > 90%

Appearance: white solid

R_f: 0.8 (1:1; *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 8.41 (s, 1H, H₇), 7.94 (d, 2H, *J* = 6.6 Hz), 6.97 (d, 2H, *J* = 10 Hz), 6.59 (s, 2H, H_{1&2}), 3.90 (s, 6H, H_{8&10}), 3.85 (s, 3H, H₉), 3.71 (q, 2H, *J* = 8 Hz, CH₂), 1.44 (t, 3H, *J* = 8.1 Hz).

¹³C NMR (100 MHz, CDCl₃): δ 159.7, 154.1, 131.7, 114.9, 98.2, 63.9, 61.0, 58.4, 56.2, 56.0, 18.4, 14.6

(*E*)-1-(4-(ethylthio)phenyl)-*N*-(3,4,5-trimethoxyphenyl)methanimine (56)

56 was synthesised from 4-methoxy-3-nitrobenzaldehyde using **General Method III, Method A**.

Yield: > 90%

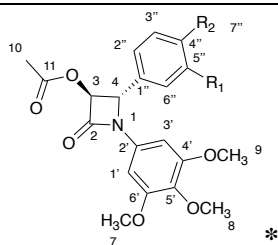
Appearance: bright orange solid

R_f: 0.67 (1:1; *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 8.42 (s, 1H, H₇), 7.84 (d, 2H, *J* = 9 Hz, H_{3&4}), 7.34 (d, 2H, *J* = 9 Hz, H_{5&6}), 6.54 (s, 2H, H_{1&2}), 3.89 (s, 6H, H_{8&10}), 3.89 (s, 3H, H₉), 3.02 (q, 2H, *J* = 9 Hz, SCH₂), 1.37 (t, 3H, *J* = 8.2 Hz, CH₃)

¹³C NMR (100 MHz, CDCl₃): δ 158.9, 153.6, 130.1, 129.4, 127.2, 126.4, 98.3, 61.0, 56.2, 26.5, 25.9, 14.1, 13.8

Numbering nomenclature for 3-acetoxy β-lactams



2-Azetidinone Code	R ₁	R ₂
16a	OTBDMS	OCH ₃
17a	H	OCH ₃
18a	F	OCH ₃
19a	Cl	OCH ₃
20a	CH ₃	OCH ₃
21a	Br	OCH ₃
22a	H	SCH ₃
65a	H	OCH ₂ CH ₃
66a	H	SCH ₂ CH ₃
89a	H	F
90a	H	NO ₂

*one enantiomer shown

2-(3-((*Tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl acetate (**16a**)³⁷⁸

16a was synthesised from **47** using **General Method IV**.

Yield: 1.46g (0.3 mmol) 60%

Melting Point: 133.4-138.9 °C

Appearance: yellow powder

R_f: 0.63 (2:1; *n*-hexane: ethyl acetate; 0.6 (1:1; *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 6.91 (dd, 1H, *J*=8.7 Hz, *J*= 2.5 Hz, H_{2''}), 6.85 (d, 1H, *J*= 8.2 Hz, H_{3''}), 6.78 (d, 1H, *J*=2.5 Hz, H_{6''}), 6.52 (s, 2H, H_{1'&3'}), 5.34 (1H, d, *J*=1.5 Hz, H₃), 4.80 (d, 1H, *J*=1.5 Hz, H₄), 3.79 (s, 3H, H₈), 3.74 (s, 3H, H_{7''}), 3.68 (s, 6H, H_{7&9}), 2.18 (s, 3H, H₁₀), 0.91 (s, 9H, *t*- butyl C(CH₃)₃), 0.06 (s, 3H, Si-CH₃), 0.04 (s, 3H, Si-CH₃)

¹³C NMR (100 MHz, CDCl₃): 169.6 (C₁₁), 161.6 (C₂), 153.4 (C_{4'&6'}), 151.6, 145.8, 142.5, 135.0, 133.0, 127.4, 119.9, 119.0, 119.0, 112.5, 95.4 (C_{1'&3'}), 82.3 (C₃), 63.6 (C₄), 62.0, 56.0, 55.4, 20.5 (C₁₀), 18.5 (C(CH₃)₃), 0.9 (Si-CH₃), -4.7 (Si-CH₃)

2-(4-Methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl acetate (**17a**)³⁷⁸

17a was synthesised from **44** using **General Method IV**.

Yield (preliminary): 926 mg (2.3 mmol) 46%, isolated as a 1:1 *trans*:*cis* isomeric mixture.

Yield (optimised) 1.16 g (2.9 mmol) 58%, 95:5 *trans*:*cis*)

Appearance: white crystalline solid

R_f: 0.41 (1:1; *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ (isomeric mixture): 7.29 (d, 2H, *J* = 8.5 Hz, H_{2''&6''}, *cis*), 7.24 (d, 2H, *J* = 8.5 Hz, H_{2''&6''}, *trans*), 6.92 (d, 2H, *J* = 8.5 Hz, H_{3''&5''}, *cis*), 6.88 (d, 2H, *J* = 8.2 Hz, H_{3''&5''}, *trans*), 6.57 (s, 2H, H_{1'&3'}, *cis*), 6.53 (s, 2H, H_{1'&3'}, *trans*), 5.89 (d, 1H, *J*= 4.5 Hz, H₃,

cis), 5.37 (d, 1H, $J = 1.8$ Hz, H₃, *trans*), 5.29 (d, 1H, $J = 4.5$ Hz, H₄, *cis*), 4.86 (d, 1H, $J = 1.8$ Hz, H₄, *trans*), 3.77 (s, 3H), 3.76 (s, 3H), 3.76 (s, 3H), 3.72 (s, 6H), 3.69 (s, 6H, H_{7&9}), 2.21 (s, 3H, H₁₀)

¹H NMR (400 MHz, CDCl₃): δ (*trans*) 7.28 (d, 2H, $J = 8.6$ Hz, H_{2'&6'}), 6.90 (d, 2H, $J = 8.6$ Hz, H_{3'&5'}), 6.52 (s, 2H, H_{1'&3'}), 5.36 (d, 1H, $J = 1.8$ Hz, H₃), 4.85 (d, 1H, $J = 1.8$ Hz, H₄), 3.80 (s, 3H, H₈), 3.79 (s, 3H, H₇), 3.75 (s, 3H, H₈), 2.03 (s, 3H, H₁₀)

¹³C (100 MHz, CDCl₃): δ (*trans*) 161.7 (C₁₁), 160.2 (C₂), 153.5 (C_{4'&6'}), 135.0, 133.0, 129.4, 128.0, 127.7, 127.0, 114.6, 95.4 (C_{1'&3'}), 82.5 (C₃), 63.7 (C₄), 61.0 (C₈), 56.0 (C_{7&9}), 55.4 (C_{7'}), 20.5 (C₁₀)

IR $\nu_{\max}$ (ATR): 1746 cm⁻¹ (β -lactam C=O)

2-(3-Fluoro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl acetate (18a)

400

18a was synthesised from **45** using **General Method IV**.

Yield: 1.22 g (2.9 mmol) 58%, 96:4 *cis:trans*)

R_f: 0.4 (1:1; *n*-hexane: ethyl acetate)

Appearance white powder

¹H NMR (400 MHz, CDCl₃): δ 7.08 (apparent d, 2H, $J = 9$ Hz, H_{6'&2'}), 6.96 (t, 1H, $J = 8.3$ Hz, H_{3'}), 6.51 (s, 2H, H_{1'&3'}), 5.31 (d, 1H, $J = 1.3$ Hz, H₃), 4.81 (d, 1H, $J = 1.3$ Hz, H₄), 3.88 (s, 3H, H_{7'}), 3.76 (s, 3H, H₈), 3.69 (s, 6H, H_{7&9}), 2.17 (s, 3H, H₁₀)

¹³C (100 MHz, CDCl₃): δ 169.7 (C₁₁), 161.4 (C₂), 153.4 (C_{4'&6'}), 132.7, 127.8, 122.2, 114.1, 113.8, 95.3 (C_{1'&3'}), 82.5 (C₃), 63.0 (C₄), 61.0, 56.3 (C_{7&9}), 55.9, 20.3 (C₁₀)

¹⁹F NMR (376 MHz, CDCl₃): δ -133.13

2-(3-Chloro-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)2-azetidin-3-yl acetate (19a)

400

19a was synthesised from **48** using **General Method IV**.

Yield: 1.63g (3.6 mmol) 72%

R_f: 0.13 (2:1; *n*-hexane:ethyl acetate)

Appearance: white solid powder

Melting Point: 115 °C

¹H NMR (400 MHz, CDCl₃): δ 7.37 (apparent s, 1H, H_{6'}), 7.20 (apparent d, 1H, $J = 8.0$ Hz, H_{2'}), 6.91 (d, 1H, $J = 8.0$ Hz, H_{3'}), 6.49 (s, 2H, H_{1'&3'}), 5.31 (apparent s, 1H, H₃), 4.80 (apparent s, 1H, H₄), 3.87 (s, 3H, H_{7'}), 3.74 (s, 3H, H₈), 3.69 (s, 6H, H_{7&9}), 2.17 (s, 3H, H₁₀)

¹³C NMR (100 MHz, CDCl₃): δ 169.6 (C₁₁), 161.5 (C₂), 155.7, 153.5 (C_{4'&6'}), 135.2, 132.7, 128.3, 128.1, 125.8, 123.4, 120.0, 118.8, 112.5, 95.3 (C_{1'&3'}), 82.4 (C₃), 63.1 (C₄), 60.9, 56.2, 56.1, 20.0 (C₁₀)

IR $\nu_{\max}$ (ATR): 1746 cm⁻¹ (β -lactam C=O)

2-(4-Methoxy-3-methylphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)2-azetidine-3-yl acetate (20a)

400

20a was synthesised from **49** using **General Method IV**.

Yield: 2.03 g (4.89 mmol) 49 %

Melting Point: 59-61 °C

R_f: 0.38 (2:1; *n*-hexane:ethyl acetate)

Appearance: yellow solid powder

¹H NMR (400 MHz, CDCl₃): δ 7.18 (dd, *J* = 8 Hz, *J* = 2.7 Hz, 1H, H_{2''}), 7.15 (d, 1H, *J* = 2.7, H_{6''}), 6.84 (d, 1H, *J* = 8 Hz, H_{3''}), 6.58 (s, 2H, H_{1'&3'}), 5.31 (d, 1H, *J* = 1.8 Hz, H₃), 4.84 (d, 1H, *J* = 1.8 Hz, H₄), 3.85 (s, 3H, H_{7''}), 3.79 (s, 3H, H₈), 3.73 (s, 6H, H_{7&9}), 2.22 (s, 3H, H₁₀), 2.21 (s, 3H, R₁, CH₃)

¹³C NMR (100 MHz, CDCl₃): δ 169.7 (C₁₁), 162.0 (C₂), 158.4, 153.51 (C_{4'&6'}), 135.1, 133.2, 128.5, 127.8, 126.6, 125.1, 110.2, 95.5 (C_{1'&3'}), 82.1, 63.8, 61.0, 56.2, 55.4, 20.6 (C₁₀), 16.0 (R₁, CH₃)

IR ν_{max} (ATR): 1749 cm⁻¹ (β-lactam C=O)

2-(4-(Methylthio)phenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)2-azetidine-3-yl acetate (22a)³⁷⁸
22a was synthesised from **50** using **General Method IV**.

Yield: 700 mg (1.79 mmol) 36 %

R_f: 0.45 (1:1; *n*-hexane:ethyl acetate) **Appearance:** white solid powder

Melting Point: 121 – 122 °C.

¹H NMR (400 MHz, CDCl₃): δ 7.29 (d, 2H, *J* = 6 Hz, H_{2''&6''}), 7.25 (d, 2H, *J* = 8 Hz, H_{3''&5''}), 6.56 (s, 2H, H_{1'&3'}), 5.38 (d, 1H, *J* = 1.9 Hz, H₃), 4.89 (d, 1H, *J* = 1.9 Hz, H₄), 3.79 (s, 3H, H₈), 3.72 (s, 6H, H_{7&9}) 2.49 (s, 3H, H_{7''}), 2.20 (s, 3H, H₁₀)

¹³C NMR (100 MHz, CDCl₃): δ 169.8 (C₂), 161.5, 153.6 (C_{4'&6'}), 140.1, 132.9, 131.67, 130.02, 129.1, 128.2, 126.89, 125.3, 125.2, 95.4 (C_{1'&3'}), 82.5 (C₄), 63.7 (C₃), 60.1, 56.1, 20.52, 15.5

IR ν_{max} (ATR): 1720 cm⁻¹ (β-lactam C=O)

2-(4-fluorophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl) 2-azetidine-3-yl acetate (89a)

89a was synthesised from **51** using **General Method IV**.

Yield: 1.03g (2.65 mmol) 30%

Appearance: yellow powder

R_f: 0.46 (1:1 *n*-hexane: ethyl acetate)

Melting Point: 52-56 °C

¹H NMR (400 MHz, CDCl₃): δ 7.37 (apparent multiplet, 2H, H_{2''&6''}), 7.12 (apparent triplet, *J* = 8.6 Hz, 2H, H_{3''&5''}), 6.52 (s, 2H, H_{1'&3'}), 5.35 (d, 1H, *J* = 1.9 Hz, H₃), 4.92 (d, 1H, *J* = 2.0 Hz, H₄), 3.79 (s, 3H, H₈), 3.72 (s, 6H, H_{7&9}), 2.22 (s, 3H, H₁₀)

¹³C NMR (100 MHz, CDCl₃): δ 166.1 (C₁₁), 164.2 (C₂), 161.7, 153.5 (C_{4'&6'}), 134.8 (C_{5'}), 133.2 (C_{2'}), 128.0, 116.4 (C_{2'&6'}), 116.2 (C_{3'&5'}), 95.5 (C_{1'&3'}), 83.9 (C₃), 65.1 (C₄), 61.0 (C₈), 56.1 (C_{7&9})

¹⁹F NMR (376 MHz, CDCl₃): δ -111.7

IR ν_{max} (ATR): 1750 cm⁻¹ (β-lactam C=O)

2-(4-Nitrophenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)2-azetidine-3-yl acetate (90a)

90a was synthesised from **52** using **General Method IV**.

Yield: 610 mg (1.48 mmol) 37%

R_f: 0.63 (*trans*), 0.54 (*cis*), (1:1; *n*-hexane:ethyl acetate)

Appearance: white solid powder

Melting Point: 66-68 °C

¹H NMR (400 MHz, CDCl₃): (mixture of *cis:trans* isomers; 44:66) δ 8.32 (d, 2H, *J* = 8.1 Hz, (*trans*), H_{3''&5''}), 8.27 (d, 1.4H, *J* = 8.6 Hz, (*cis*) H_{3''&5''}), 7.59 (d, 2H, *J* = 8.1 Hz, (*trans*) H_{2''&6''}), 7.54 (d, 1.4H, *J* = 8.6 Hz, (*cis*) H_{2''&6''}), 6.54 (s, 1.4H, (*cis*) H_{1'&3'}), 6.49 (s, 2H, (*trans*) H_{1'&3'}), 6.03 (d, 0.44H, *J* = 4.7 Hz, (*cis*), H₃), 5.49 (d, 0.44H, *J* = 4.7 Hz, (*cis*), H₃), 5.29 (d, 0.66H, *J* = 1.7 Hz,

(*trans*), H₄), 5.04 (d, 0.66H, *J* = 1.7 Hz, (*trans*) H₄), 3.80 (s, 1.6H, (*cis*), H₈), 3.79 (s, 3H, (*trans*), H₈), 3.73 (s, 6H, (*trans*), H_{7&9}), 2.25 (s, 3H, H₁₀)

¹³C NMR (100 MHz, CDCl₃): δ 164.0 (C₁₀), 161.9 (C₂), 153.8 (C_{4'&6'}), 142.3, 132.3, 128.9, 127.4, 124.5, 123.9, 116.2, 100.2, 95.4 (C_{1'&3'}, *trans*), 95.1 (C_{1'&3'}, *cis*), 85.9 (C₃, *trans*), 82.3 (C₃, *cis*), 63.1 (C₄), 61.0, 60.9, 56.2, 56.1, 20.5 (C₁₀, *trans*), 19.9 (C₁₀, *cis*)

IR ν_{max} (ATR): 1751 cm⁻¹ (β -lactam C=O), 1506.2 cm⁻¹ (N-O)

2-(3-Bromo-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)2-azetidine-3-yl acetate (21a)

21a was synthesised from **53** using **General Method IV**.

Yield: 2.39g (4.79 mmol) 63%

Melting Point: 98-101 °C

R_f: 0.45 (1:1; *n*-hexane:ethyl acetate)

Appearance white solid powder

¹H NMR (400 MHz, CDCl₃): δ 7.57 (apparent s, 1H, H_{6''}), 7.27 (d, 1H, *J* = 8 Hz, H_{3''}), 6.91 (d, 1H, *J* = 8 Hz, H_{2''}), 6.52 (s, 1H, H_{1'&3'}), 5.34 (apparent singlet, 1H, H₃), 4.81 (apparent singlet, 1H, H₄), 3.91 (s, 3H, H_{7''}), 3.78 (s, 3H, H₈), 3.71 (s, 6H, H_{7&9}), 2.16 (s, 3H, H₁₀)

¹³C NMR (100 MHz, CDCl₃): δ 169.7 (C₁₀), 161.3 (C₂), 156.6 (H_{4'&6'}), 153.3, 135.9, 132.7, 131.4, 128.4, 126.8, 112.5, 95.7 (C_{1'&3'}), 82.6 (C₃), 65.8 (C₄), 62.8, 60.8, 56.5 (C_{7&9}), 20.9 (C₁₀)

IR ν_{max} (ATR): 1760 cm⁻¹ (β -lactam C=O)

4-(4-Ethoxyphenyl)-3-(methylperoxy)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (65a)

65a was synthesised from **55** using **General Method IV**.

Yield: 0.91g (2.25 mmol) 45 %

R_f: 0.5 (1:1; *n*-hexane:ethyl acetate)

Appearance: off white solid

¹H NMR (400 MHz, CDCl₃): δ 7.27 (d, 2H, *J* = 8.8 Hz, H_{2''&6''}), 6.90 (d, 2H, *J* = 8.8 Hz, H_{2''&3''}), 6.53 (s, 2H, H_{1'&3'}), 5.37 (apparent singlet, 1H, H₃), 4.85 (apparent singlet, 1H, H₄), 4.01 (q, 2H, *J* = 6.3 Hz, OCH₂), 3.76 (s, 3H, H₈), 3.69 (s, 6H, H_{7&9}), 2.12 (s, 3H, H₁₀), 1.40 (t, 3H, *J* = 6.3 Hz CH₃ of OCH₂CH₃)

¹³C NMR (100 MHz, CDCl₃): δ 169.8, 161.8, 159.6, 153.5, 133.1, 129.1, 127.7, 126.9, 114.9, 95.2, 82.4, 63.8, 63.5, 61.0, 56.0, 20.4, 14.8

4-(4-Ethylthio)phenyl)-3-(methylperoxy)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (66a)

66a was synthesised from **56** using **General Method IV**.

Yield: 0.86 g (2.05 mmol) 40%

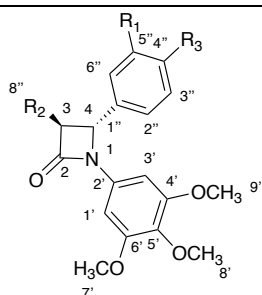
R_f: 0.5 (1:1; *n*-hexane:ethyl acetate)

Appearance: yellow solid

¹H NMR (400 MHz, CDCl₃): δ 7.32 (d, 2H, *J* = 8.5 Hz, H_{2''&6''}), 7.3 (d, 2H, *J* = 8.5 Hz, H_{3''&5''}), 6.54 (s, 2H, H_{1'&3'}), 5.39 (d, 1H, *J* = 1.8 Hz, H₃), 4.89 (d, 1H, *J* = 1.8 Hz, H₃), 3.78 (s, 3H, H₈), 3.72 (s, 6H, H_{7&9}), 2.97 (q, 2H, *J* = 7.4 Hz, S-CH₂), 2.21 (s, 3H, H₁₀), 1.34 (t, 3H, *J* = 7.8 Hz, CH₃ of S-CH₂CH₃)

¹³C NMR (100 MHz, CDCl₃): δ 169.7, 161.4, 153.5, 140.6, 138.4, 135.1, 132.8, 132.2, 129.0, 126.9, 95.4, 82.4, 63.8, 61.0, 56.1, 27.1, 20.6, 14.3

Numbering nomenclature for 3-substituted β -lactams

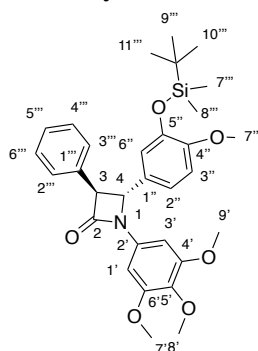


Code	R ₁	R ₂	R ₃
70	OTBDMS		OCH ₃
71	OTBDMS		OCH ₃
91	H		NO ₂
72	OTBDMS		OCH ₃
36	NO ₂		OCH ₃
73	OTBDMS		OCH ₃
74	OTBDMS		OCH ₃
75	OTBDMS		OCH ₃
31	NO ₂		OCH ₃
34	NO ₂		OCH ₃

*one enantiomer shown

4-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-phenyl-1-(3,4,5-trimethoxyphenyl)-2-azetidine-2-one (70)³⁷⁸

70 was synthesised with 47 and phenylacetyl chloride using General Method V.



Yield: 878 mg (1.6 mmol) 32%

R_f: 0.2 (3:1 *n*-hexane: ethyl acetate); 0.55 (2:1 *n*-hexane: ethyl acetate)

Appearance: yellow powder

Melting Point: 297 °C

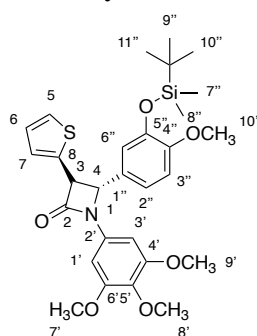
¹H NMR (400 MHz, CDCl₃): δ 7.32 (m, 5H, H_{2''''}-H_{6''''}), 6.95 (dd, 1H, *J*=8.5 Hz, *J*=2.1 Hz, H_{2''}), 6.85 (m, 2H, H_{3''} & H_{6''}), 6.58 (s, 2H, H_{1'&3'}), 4.78 (d, 1H, *J*=2 Hz, H₃), 4.24 (d, 1H, *J*=2 Hz, H₄), 3.79 (s, 3H, H_{7''}), 3.75 (s, 3H, H_{8'}), 3.70 (s, 6H, H_{7'&9'}), 0.91 (s, 9H, *t*-butyl, H_{9''''}, 10'''' & 11'''), 0.07 (s, 3H, Si-CH₃), 0.05 (s, 3H, Si-CH₃)

¹³C NMR (100 MHz, CDCl₃): δ 165.4 (C₂), 153.4 (C_{4'&6'}), 151.3, 145.7, 134.8, 133.6, 129.7, 129.4, 128.8, 128.3, 127.8, 127.4, 119.5, 118.4, 112.4, 94.9 (C_{1'&3'}), 64.9 (C₃), 63.7 (C₄), 55.9, 55.5, 25.6, -4.7 (Si-CH₃), -4.8 (Si-CH₃)

IR ν_{max} (ATR): 1748 cm⁻¹ (β-lactam C=O)

4-(4-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl) azetidine-2-one (71)

71 was synthesised from 2-thiopheneacetylchloride and **47** using **General Method V**.



Yield: 1.58 g (2.92 mmol) 58 %

Appearance: pink/red powder

R_f: 0.54 (1:1 *n*-hexane: ethyl acetate)

Melting Point: 51-55 °C

¹H NMR (400 MHz, CDCl₃): δ 7.31 (dd, 1H, *J*= 5.63 Hz, *J*= 1.3 Hz, H₆), 7.10 (d, 1H, *J*= 3.1 Hz, H₇), 7.09 (dd, 7.09, 1H, *J*= 8 Hz, 1.9Hz, H₅), 6.99 (dd, 1H, *J*=8.3 Hz, *J*= 2.1 Hz, H_{2''}), 6.90 (d, 1H, *J*= 8.9 Hz, H_{3''}), 6.89 (d, 1H, *J*= 1.8 Hz, H_{6''}), 6.61 (s, 2H, H_{1'&3'}), 4.86 (d, 1H, *J*= 1.8 Hz, H₄), 4.45 (d, 1H, *J*= 1.8 Hz, H₃), 3.84 (s, 3H, H_{7''}), 3.79 (s, 3H, H_{8'}), 3.73 (s, 6H, H_{7'&9'}), 0.96 (s, 9H, *t*-butyl, H_{9''''}, 10'''' & 11'''), 0.12 (s, 3H, Si-CH₃), 0.09 (s, 3H, Si-CH₃)

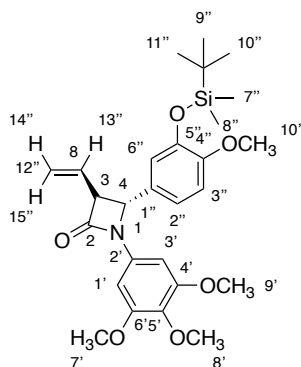
¹³C NMR (100 MHz, CDCl₃): δ 164.3 (C₂), 153.5 (C_{4'&6'}), 151.4 (C_{4''}), 145.8 (C_{5''}), 136.2 (C₈), 134.6 (C_{2'}), 133.6 (C_{5'}), 129.2 (C_{1''}), 127.2 (C₆), 125.7 (C₅), 125.3 (C₇), 119.4 (C_{2''}), 118.4 (C_{6''}), 112.5 (C_{3''}), 95.04 (C_{1'&3'}), 64.5 (C₄), 60.9 (C_{8'}), 60.1 (C₃), 56.0 (C_{7'&9'}), 25.6 (C_{9''}, 10'' & 11'''), -4.7, -4.7 (Si-CH₃)

IR ν_{max} (ATR): 1751 cm⁻¹ (β-lactam C=O)

LRMS: APCI calculated for [M+H⁺] 556 m/z; found 556.11

4-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-one (72)

72 was synthesised from crotonyl chloride and **47** using **General Method V**.



Yield: not calculated. (De-silylated prior to purification)

Appearance: orange gel

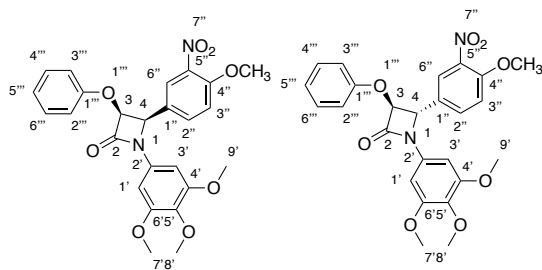
R_f: 0.7 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 6.96 (dd, 1H ³*J* = 2.2 Hz, ³*J* = 8 Hz, H_{2''}), 6.87 (d, 1H ³*J* = 8 Hz, H_{3''}), 6.83 (d, 1H, ³*J* = 2.1 Hz, H_{6''}), 6.57 (s, 2H, H_{1'&3'}), 6.04 (td, 1H, ³*J*_{13''-15''} = 17.1 Hz, ³*J*_{13''-14''} = 9.9 Hz, ³*J*_{13''-3} = 2.2 Hz, H_{13''}), 5.40 (dd, 1H, ³*J*_{15''-13''} = 16 Hz, ²*J*_{15''-14''} = 2.2 Hz, H_{15''}), 5.34 (dd, 1H, ³*J*_{14''-13''} = 9.9 Hz, ²*J*_{14''-15''} = 2.2 Hz, H_{14''}), 4.69 (d, 1H, ³*J* = 2.2 Hz, H₄), 3.83 (s, 3H, H₈), 3.80 (s, 3H, H₁₀), 3.75 (apparent multiplet, 1H, H₃), 3.73 (s, 6H, H_{7'&9'}), 0.96 (s, 3H, *t*-butyl, H_{9'',10''&11''}), 0.1 (s, 3H, H_{7''}), 0.09 (s, 3H, H_{8''})

¹³C NMR (100 MHz, CDCl₃): δ 165.3 (C₂), 153.5 (C_{4'&6'}), 151.3 (C_{4''}), 145.7 (C_{5''}), 134.4 (C_{2'}), 133.8 (C_{5'}), 130.64 (C₈), 129.64 (C_{12'}), 119.8 (C_{2''}), 119.8 (C_{1''}), 112.4 (C_{3''}), 94.8 (C_{2&3}), 65.9 (C₄), 65.3 (C₃), 61.3 (C_{1''}), 60.9, 60.4 (C_{8'}), 56.0 (C_{7'&9'}), 55.5 (C_{10'}), 42.0, 30.1, 29.1, 25.6, 23.4, 23.1, 18.5, 15.3, -4.7 (Si-CH₃)

4-(4-methoxy-3-nitrophenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidine-2-one (**36**)

36 was synthesised from **54** and benzyloxyacetyl chloride using **General Method V** as an isomeric mixture. **36** *cis* and *trans* isomers were purified using LC over silica gel with an *n*-hexane:ethyl acetate gradient.



Yield (isomeric mixture): 1.2g (2.5 mmol) 36 %

Yield (isolated *cis* isomer): 460 mg (0.95 mmol) 19%

R_f: 0.13 (*cis*), 0.25 (*trans*) (1:1 *n*-hexane: ethyl acetate)

Yield: 620 mg (1.29 mmol), 26% (*cis*)

Melting Point: 150 °C solid, 71-75 °C amorphous powder (*cis*)

¹H NMR (400 MHz, CDCl₃): δ (*cis*) 7.94 (d, 1H, *J* = 2.5 Hz, H_{6''}), 7.62 (dd, 1H, *J* = 9.6 Hz, 2.5 Hz, H_{2''}), 7.22 (t, 2H, *J* = 7.8 Hz), 7.01 (d, 1H, *J* = 9.6 Hz, H_{6''}), 6.97 (t, 1H, *J* = 7.0 Hz, H_{5''}), 6.83 (d, 2H, *J* = 7.4 Hz), 6.62 (s, 2H, H_{1'&3'}), 5.6 (d, 1H, *J* = 5.2 Hz, H₃), 5.41 (d, 1H, *J* = 5.2 Hz, H₄), 3.96 (s, 3H, H₈), 3.81 (s, 3H, H_{7''}), 3.78 (s, 6H, H_{7'&9'})

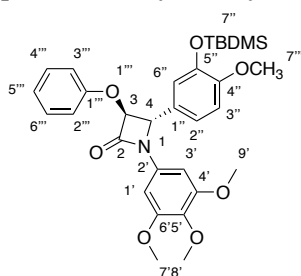
¹H NMR (400 MHz, CDCl₃): δ (*trans*) 7.95 (d, 1H, J = 3.5 Hz, H_{6''}), 7.60 (dd, 1H, J = 9.6 Hz, 2.6 Hz, H_{2''}), 7.3 (apparent multiplet, 2H, J = 7.8 Hz, H_{4'''&6'''}), 7.21 (d, 1H, J = 9.6 Hz, H_{3''}), 6.98 (t, 1H, J = 7.0 Hz, H_{5'''}), 6.84 (d, 2H, J = 7.4 Hz, H_{2'''&3'''}), 6.55 (s, 2H, H_{1'&3'}), 5.14 (d, 1H, J = 1.7 Hz, H₃), 5.04 (d, 1H, J = 1.7 Hz, H₄), 4.03 (s, 3H, H₈), 3.81 (s, 3H, H_{7'''}), 3.76 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz, CDCl₃): δ (*cis*) 162.4, 156.6, 153.8, 139.2, 135.4, 133.6, 132.6, 129.7, 125.8, 125.2, 122.6, 115.5, 113.8, 95.3, 80.9, 61.0, 60.9, 56.7, 56.2

IR ν_{max} (ATR): 1749 cm⁻¹ (β -lactam C=O), 1622.52 cm⁻¹ (N-O).

4-(3-(((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)-2-azetidine-2-one (73)

73 was synthesised from **47** and benzyloxyacetyl chloride using **General Method V** in predominantly *trans* yield (90:10 *trans*:*cis*).



Yield: 1.05g (1.9 mmol) 37 %

Appearance: white fluffy powder (90:10% *trans*: *cis*)

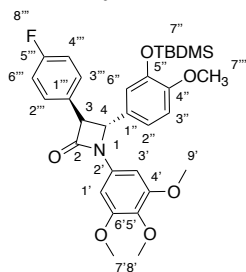
R_f: 0.7 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 7.0-6.48 (m, 8H, H_{2'''&3'''} & H_{3'',4'',6''}), 6.56 (s, 2H, H_{1'&3'}), 5.07 (apparent singlet, 1H, H₃), 4.87 (apparent singlet, 1H, H₄), 3.88 (s, 3H, H_{7'''}), 3.76 (s, 3H, H₈), 3.69 (s, 6H, H_{7'&9'}), 0.93 (s, 9H, *t*-butyl), 0.09 (s, 3H, Si-CH₃) 0.06 (s, 3H, Si-CH₃)

¹³C NMR (100 MHz, CDCl₃): δ 162.5, 157.0, 153.5, 151.8, 145.8, 135.0, 133.1, 130.2, 129.7, 127.8, 126.3, 122.3, 115.4, 112.6, 111.2, 95.5, 87.1, 65.9, 64.0, 61.0, 56.0, 55.6, 25.8, -4.8 (Si-CH₃), -4.7 (Si-CH₃)

4-(3-(((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-(4-fluorophenyl)-1-(3,4,5-trimethoxyphenyl)-2-azetidine-2-one (74)

74 was synthesised using **General Method V** from **47** and 4-fluorophenylacetyl chloride.



Yield: 1.2g (2.11 mmol) 42%

R_f: 0.65 (1:1 *n*-hexane: ethyl acetate), 0.45 (3:1 *n*-hexane: ethyl acetate)

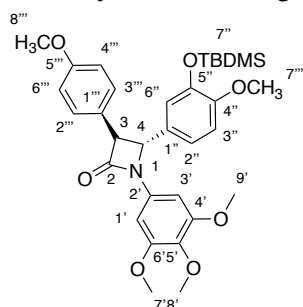
¹H NMR (400 MHz, CDCl₃): δ 7.30 (m, 2H, H_{4'''&6'''}), 7.07 (m, 2H, H_{2'''&3'''}), 6.96 (d, 1H, J = 8.6 Hz, H_{3''}), 6.87 (d, 1H, J = 7.7 Hz, H_{3''}), 6.88 (d, 1H, J = 2.6 Hz, H_{6''}), 6.59 (s, 2H, H_{1'&3'}), 4.75 (d, J = 2.1 Hz, H₃), 4.28 (s, J = 2.1 Hz, H₄), 3.81 (s, 3H, H_{7'''}), 3.77 (s, 3H, H₈), 3.71 (s, 6H, H_{7'&9'}), 0.94 (s, 9H, *t*-butyl), 0.09 (s, 3H, Si-CH₃), 0.07 (s, 3H Si-CH₃)

¹⁹F NMR (376 MHz, CDCl₃): δ -114.1

Appendices

4-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl) azetidine-2-one (75)

75 was synthesised using **General Method V** from **47** and 4-methoxyphenylacetyl chloride.



Yield: 2.34 g (3.66 mmol) 57%

Appearance: yellow/white powder

Melting Point: 121-123 °C

R_f: 0.48 (2:1 *n*-hexane: ethyl acetate), 0.58 (1:1 *n*-hexane: ethyl acetate),

Melting Point: 121-124 °C

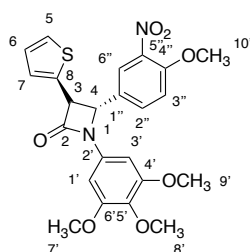
¹H NMR (400 MHz, CDCl₃): δ 7.27 (d, 2H, *J* = 9 Hz, H_{2'''}&3'''), 6.97 (dd, 1H, *J* = 8 Hz, *J* = 1.9 Hz, H_{2''}), 6.93 (d, 2H, *J* = 7.3 Hz, H_{4'''}&6'''), 6.89 (d, 1H, *J* = 8 Hz, H_{3''}), 6.87 (d, 1H, *J* = 2.19 Hz, H_{6''}), 6.62 (s, 2H, H_{7'}&9'), 4.76 (d, 1H, *J* = 2.2 Hz, H₄), 4.22 (d, 1H, *J* = 2.2 Hz, H₃), 3.84 (s, 3H, H_{8'''}), 3.81 (s, 3H, H_{7'''}), 3.79 (s, 3H, H_{8'}), 3.74 (s, 6H, H_{7'}&9'), 0.96 (s, 9H, *t*-butyl), 0.1 (s, 3H, Si-CH₃), 0.1 (s, 3H Si-CH₃).

¹³C NMR (100 MHz, CDCl₃): δ 166.1 (C₂), 159.4 (C_{5'''}), 153.5 (C_{4'}&6'), 151.4 (C_{4''}), 145.8 (C_{5''}), 134.4 (C_{5'}), 133.8 (C_{2'}), 129.9 (C_{1'''}), 128.6 (C_{2'''}&3'''), 126.9 (C_{1''}), 119.5 (C_{2''}), 118.7 (C_{6''}), 114.5 (C_{4'''}&6'''), 112.6 (C_{3''}), 95.1 (C_{1'}&3'), 65.9, 64.5 (C₃), 64.2 (C₄), 60.9 (C_{8'}), 56.0 (C_{7'}&9'), 55.7 (C_{8'''}), 55.4 (C_{7'''}), 25.6 (*t*-butyl), -4.7 (Si-CH₃)

IR ν_{max} (ATR): 1739 cm⁻¹ (β-lactam C=O)

4-(4-methoxy-3-nitrophenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl) azetidine-2-one (31)

31 was synthesised from **53** and 2-thiophenyl acetyl chloride using **General Method V** and as an isomeric mixture 9:1 *cis:trans*.



Yield: 1.51g (3.19 mmol) 40 %

Appearance yellow/white powder

R_f: 0.33 (1:1 *n*-hexane: ethyl acetate)

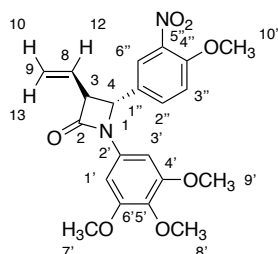
¹H NMR (400 MHz, CDCl₃): δ 8.30 (d, 0.09 H, *cis*, *J* = 1.9 Hz, H_{3''}), 8.09 (dd, 0.09H, *cis*, *J* = 2.4, Hz, *J* = 8.8 Hz, H_{2''}), 7.94 (d, 1H, *J* = 1.9 Hz, H_{7''}), 7.72 (apparent s, 0.09H, *cis*, H_{7''}), 7.62 (dd, 1H, *J* = 2.4 Hz, *J* = 8.8 Hz, H_{6''}), 7.34 (d, 1H, *J* = 5.5 Hz, H₅), 7.18 (d, 1H, *J* = 9.5 Hz, H_{3''}), 7.10 (d, 1H, *J* = 3 Hz, H₇), 7.05 (t, 1H, *J* = 4 Hz, H₆), 6.57 (s, 2H, H_{1'}&3'), 5.39 (d, 0.09H, *cis*, *J* =

3.7 Hz, H₄), 5.18 (d, 0.09H, *cis*, *J* = 3.7 Hz, H₃), 4.98 (d, 1H, *J* = 2.5 Hz, H₄), 4.49 (d, 1H, *J* = 2.5 Hz), 4.09 (s, 3H, H_{10'}), 3.80 (s, 3H, H_{8'}), 3.77 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz, CDCl₃): δ 163.8 (C₂), 153.8 (C_{4'&6'}), 153.3 (C_{4''}), 140.1 (C_{5''}), 135.2 (C₈), 135.1 (C_{5'}), 133.1 (C_{2'}), 131.2 (C_{2''}), 129.3 (C_{1''}), 127.5 (C₆), 126.1 (C₇), 125.7 (C₅), 123.6 (C_{6''}), 114.7 (C_{3''}), 95.0 (C_{1'&3'}), 63.4 (C₄), 61.0 (C_{8'}), 60.2 (C₃), 56.8 (C_{10'}), 56.2 (C_{7'&9'})

4-(4-methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-one (34)³⁵⁹

34 was synthesised from **47** and crotonyl chloride using **General Method V**.

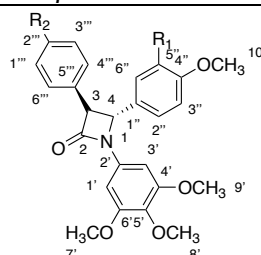


Yield: 480 mg (1.15 mmol) 11%

R_f: 0.25 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 7.91 (d, 1H, *J* = 2.9 Hz, H_{6''}), 7.56 (dd, 1H, *J* = 1.9, *J* = 8.1 Hz, H_{2''}), 7.27 (d, 1H, *J* = 8 Hz, H_{3''}), 6.54 (s, 2H, H_{1'&3'}), 6.05 (td, 1H, ²*J* = 17 Hz, ³*J* = 2.6 Hz, ²*J* = 8.6 Hz, H₁₂), 5.41 (apparent multiplet, 2H, H_{10&13}), 4.81 (d, 1H, *J* = 2.1 Hz, H₄), 4.01 (s, 3H, H_{10'}), 3.88 (apparent multiplet, 1H, H₃), 3.81 (s, 3H, H_{8'}), 3.77 (s, 6H, H_{7'&9'})

Numbering nomenclature for 3-phenolic β-lactams



3-benzyloxy code	R ₁	R ₂
79a	OTBDMS	OBn
80	F	OBn
81	H	OBn
43	OTBDMS	NH-CBZ

3-(4-(Benzyloxy)phenyl)-4-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-2-azetidine-2-one (79a)

79a was synthesised from (4-benzyloxyphenyl)acetic acid and **47** using **General Method VI**.

Yield: 1.9g (2.9 mmol) 29 %

Melting Point: 98-103 °C

R_f: 0.31 (2:1 *n*-hexane: ethyl acetate), 0.75 (1:1 *n*-hexane: ethyl acetate) **Appearance:** white solid.

¹H NMR (400, MHz CDCl₃): δ 7.37 (m, 5H), 7.23 (d, 2H, *J* = 9 Hz), 6.95 (apparent triplet, 3H, *J* = 8.8 Hz), 6.84 (d, 2H, *J* = 6.8 Hz), 6.58 (s, 2H, H_{1'&3'}), 5.05 (s, 2H, CH₂ (benzyl)), 4.72 (d, 1H, *J* = 1.9 Hz, H₃), 4.17 (d, 1H, *J* = 1.9 Hz, H₄), 3.84 (s, 3H, H_{10'}), 3.75 (s, 3H, H_{8'}), 3.7 (s, 6H, H_{7'&9'}), 0.92 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H)

¹³C NMR (100, MHz CDCl₃): δ 176.15, 166.0, 158.4, 158.0, 153.5, 151.3, 145.7, 136.9, 136.8, 134.4, 133.7, 130.4, 129.8, 128.6, 128.6, 127.9, 127.4, 127.4, 127.1, 125.6, 119.5, 118.5, 115.4, 115.0, 112.5, 94.9, 70.0, 70.0, 64.4, 64.1, 60.9, 60.9, 56.0, 55.5, 39.9, 25.6, 18.4, -4.7, -4.8
IR ν_{max}(ATR): 1740 cm⁻¹ (β-lactam C=O)

3-(4-(benzyloxy)phenyl)-4-(3-fluoro-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-2-azetidine-2-one (80)

80 was synthesised from (4-benzyloxyphenyl)acetic acid and **45** using **General Method VI**

Yield: 820mg (1.55 mmol) 24 %

Appearance: Grey amorphous powder

Melting Point: 60-63 °C

R_f: 0.54 (1:1 *n*-hexane: ethyl acetate), 0.75 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (600 MHz, DMSO-*d*₆): δ 7.42 (m, 5H), 7.33 (d, 1H, *J* = 8.3 Hz), 7.26 (d, 2H, *J* = 8.3 Hz), 7.20 (t, 1H, *J* = 8.3 Hz), 7.05 (d, 2H, *J* = 8.3 Hz), 6.60 (d, 2H, H_{1'&3'}), 5.19 (d, 1H, *J* = 2 Hz, H₃), 5.12 (s, 2H, benzyl CH₂), 4.22 (d, 1H, *J* = 2 Hz, H₄), 3.83 (s, 3H, H_{10'}), 3.65 (s, 6H, H_{7'&9'}), 3.58 (s, 3H, H_{8'})

¹³C NMR (100 MHz, DMSO-*d*₆): δ 166.1 (C₂), 158.4 (C_{4''}), 153.6 (C_{4'&6'}), 152.7, 151.3, 147.5, 137.5, 134.5, 133.7, 133.9, 130.9, 130.7, 129.25, 128.9, 128.27, 128.1, 127.5, 123.9, 115.7, 115.1, 114.8, 114.7, 95.6 (C_{1&3}), 69.6, 63.4 (C₄), 62.15 (C₃), 60.6 (C_{8'}), 60.2, 56.5 (C_{7'&9'}), 56.2 (C_{10'})

¹⁹F NMR: (376 MHz, DMSO-*d*₆): δ -134.8

IR ν_{max}(ATR): 1746 cm⁻¹ (β-lactam C=O)

3-(4-(benzyloxy)phenyl)-4-(4-methoxyphenyl)-1-(3,4, 5-trimethoxyphenyl)-2-azetidine-2-one (81)

81 was synthesised from (4-benzyloxyphenyl)acetic acid and **44** using **General Method VI**

Yield: not calculated

R_f: 0.54 (2:1 *n*-hexane: ethyl acetate), 0.75 (1:1 *n*-hexane: ethyl acetate)

Appearance: White solid.

¹H NMR (600 MHz, CDCl₃): δ 7.45 (d, 2H, *J* = 6.8 Hz), 7.41 (t, 2H, *J* = 7.3 Hz), 7.34 (d, 2H, 8.5 Hz, H_{3'&5'}), 7.27 (d, 2H, *J* = 8.4 Hz), 6.99 (d, 2H, *J* = 8.4 Hz), 6.95 (d, 2H, *J* = 7.8 Hz, H_{2'&6'}), 6.61 (s, 2H, H_{1&3}), 5.09 (s, 2H, benzyl CH₂), 4.82 (d, 1H, *J* = 3 Hz), 4.23 (d, 1H, *J* = 3 Hz), 3.84 (s, 3H, H₁₀), 3.79 (s, 3H, H₈), 3.74 (s, 6H, H_{7&9})

¹³C NMR (100 MHz, CDCl₃): δ 166.4 (C₂), 160.2 (C_{4'}), 158.7 (C_{4''}), 153.6 (C_{4'&6'}), 137.2, 134.7 (C_{5''}), 134.0, 130.8, 129.7, 128.9, 128.0, 125.8, 115.8, 114.9, 95.4 (C_{1'&3'}), 70.2 (Benzyl CH₂), 64.2, 61.0 (C_{8'}), 56.3 (C_{7'&9'}), 58.8 (C_{10'})

Benzyl (4-(2-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl) azetidine-3-yl)phenyl)carbamate (43)

43 was synthesised from (4-benzyloxycarbonylaminophenyl) acetic acid and **47** using **General Method VI**.

Yield: 470 mg (0.67 mmol) 37% yield

R_f: 0.63 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (600 MHz, CDCl₃): δ 7.43-7.34 (m, 7H, CBZ Ar-H, H_{1''''&3''''}), 7.29 (d, 2H, *J* = 8.7 Hz, H_{4''''&6''''}), 6.97 (dd, 1H, *J* = 1.9, *J* = 8.6 Hz, H_{2''}), 6.89 (d, 1H, *J* = 8.3 Hz, H_{3''}), 6.87 (d, 1H, *J* = 1.8 Hz, H_{6''}), 6.74 (bs, 1H, NH), 6.61 (s, 2H, H_{1'&3'}), 5.23 (s, 2H, CH₂ of CBZ group), 4.76 (d,

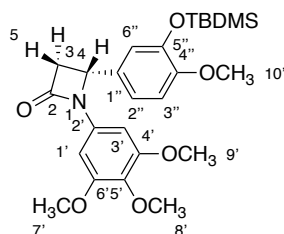
1H, $J = 2.6$ Hz, H₃), 4.23 (d, 1H, $J = 2.6$ Hz, H₄), 3.84 (s, 3H, H₁₀), 3.8 (s, 3H, H₈), 3.7 (s, 6H, H_{7&9}), 0.9 (s, 9H, *t*-butyl), 0.1 (s, 3H, Si-CH₃), 0.1 (s, 3H, Si-CH₃)

Numbering nomenclature for Reformatsky products

Code	R
76	H
77 trans	CH ₃
77 cis	CH ₃
78	F

(S)-4-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidine-2-one (76)³⁸⁴

76 was synthesised from **47** and ethyl bromoacetate using **General Method VII A** and **General Method VII B**.



Yield using reflux: 1.05 g (2.8 mmol) 40 %

Yield using microwave: 403 mg (0.85 mmol) 22%

Appearance: Yellow brown oil

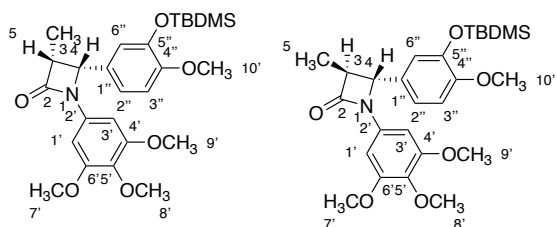
R_f: 0.78 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 6.95 (d, 1H, $J_{6'-2'} = 2.7$ Hz, H_{6''}), 6.89 (dd, 1H, $J_{2''-3''} = 9.3$ Hz, $J_{2''-6''} = 2.7$ Hz, H_{2''}), 6.86 (d, 1H, $J_{3''-2''} = 8.3$ Hz, H_{3''}), 6.56 (s, 2H, H_{1'&3'}), 4.88 (dd, 1H, $J_{4-5} = 5.5$ Hz, $J_{4-3} = 15$ Hz, H_{4'}), 3.82 (s, 3H, H₁₀), 3.77 (s, 3H, H₈), 3.72 (s, 6H, H_{7&9}), 3.52 (dd, 1H, $J_{3-5} = 14.9$ Hz, $J_{3-4} = 5.5$ Hz, H₃), 2.93 (dd, 1H, $^2J_{5'-3'} = 15$ Hz, $J_{4-3} = 2.1$ Hz, H₅), 0.96 (s, 9H, *t*-butyl), 0.1 (s, 3H, Si-CH₃), 0.08 (s, 3H, Si-CH₃)

¹³C NMR (100 MHz, CDCl₃): δ 164.6 (C₂), 153.5 (C_{4'&6'}), 151.2 (C_{5''}), 145.7 (C_{4''}), 134.3 (C_{2'}), 130.5 (C_{5'}), 119.4, 118.5 (C_{2''}), 112.2 (C_{6''}), 110.9 (C_{3''}), 95.6 (C_{1'&3'}), 60.9 (C_{8'}), 56.0 (C_{7'&9'}), 55.3 (C₁₀), 54.1 (C₄), 46.8 (C₄), 25.6 (*t*-butyl), -4.7 (Si-CH₃)

(4-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidine-2-one (77)³⁸⁴

The isomeric mixture of **77** was synthesised **47** and ethyl bromopropionate using **General Method VII A** and **General Method VII B**.



Yield using microwave: 1.7g (3.49 mmol) 39%

Yield using reflux: 1.34g (2.75 mmol) 63%

R_f: 0.9 (1:1 *n*-hexane: ethyl acetate), 0.7 (2:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 6.95 (dd, 1H, $J_{2''-3''} = 2.8$ Hz, $J_{2''-6''} = 8.3$ Hz, H_{2''}), 6.86 (d, 1H, $J_{3''-2''} = 8.3$ Hz, H_{3''}), 6.83 (d, 1H, $J_{6''-2''} = 1.9$ Hz, H_{6''}), 6.71 (d, 0.6H, $J_{6''-2''} = 1.9$ Hz, H_{6'''}), 6.58 (s, 1.4 H, H_{1'&3'}), 6.56 (s, 0.55H, H_{1'&3'}), 5.07 (d, 0.7H, $J_{4-3} = 4.8$ Hz, *cis*, H₄), 4.45 (d, 0.25H, $J_{4-3} = 1.9$ Hz, *trans* H₄), 3.8 (s, 3H, H_{10'}), 3.76 (s, 2H, H_{8'}), 3.75 (s, 1H, H_{8'}), 3.71 (s, 4H, H_{7'&9'}), 3.7 (s, 2H, H_{7'&9'}), 3.61 (apparent multiplet, 1H, H₃), 3.09 (dq, 0.3H, $J_{3-CH_3} = 7.32$ Hz, $J_{3-4} = 1.9$ Hz, H₃), 1.49 (d, 3H, $J = 8.1$ Hz, H₅) 0.94 (s, 2H, TBDMS, *t*-butyl), 0.92 (s, 7H, TBDMS, *t*-butyl), 0.07 (s, 3H, Si-CH₃), 0.03 (s, 2H, Si-CH₃)

¹³C NMR (100 MHz, CDCl₃): δ 168.4, 153.4, 151.2, 145.6, 134.1, 133.9, 130.2, 120.5, 119.8, 118.5, 112.3, 112.0, 94.9, 94.6, 62.7, 61.0, 58.3, 56.0, 55.9, 55.5, 55.4, 55.1, 25.6, 18.5, 13.1, -4.8

(4-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidine-2-one (77 *trans*))

77 *trans* was isolated from the mixture of **77** isomers using gravity LC and isocratic conditions of 2:1 *n*-hexane: ethyl acetate.

Yield of *trans* isomer isolated from mixture: 87mg (0.18 mmol) 5%.

R_f: 0.69 (2:1 *n*-hexane: ethyl acetate)

¹H NMR (600 MHz, CDCl₃): δ 6.95 (dd, 1H, $J_{2''-6''} = 2.2$ Hz, $J_{2''-3''} = 8.34$ Hz, H_{2''}), 6.86 (d, 1H, $J_{3''-2''} = 8.34$ Hz, H_{3''}), 6.83 (d, 1H, $J_{6''-2''} = 1.9$ Hz, H_{6''}), 6.56 (s, 2H, H_{1'&3'}), 4.46 (d, $J_{4-3} = 1.9$ Hz, 1H, H₄), 3.83 (s, 3H, H_{10'}), 3.79 (s, 3H, H_{8'}), 3.73 (s, 6H, H_{7'&9'}), 3.12 (dq, $J_{3-CH_3} = 7.3$ Hz, $J_{3-4} = 1.9$ Hz, H₃), 1.49 (d, 3H, $J_{CH_3-3} = 8.1$ Hz, H₅), 0.96 (s, 9H, *t*-butyl), 0.12 (s, 3H, Si-CH₃), 0.11 (s, 3H, Si-CH₃)

¹³C NMR (100 MHz, CDCl₃): δ 168.39 (C₂), 153.5 (C_{4'&6'}), 151.2 (C_{5''}), 145.7 (C_{4''}), 134.3 (C₇), 134.0 (C_{5'}), 130.3, 127.1 (C_{1''}), 119.7 (C_{2''}), 118.5 (C_{6''}), 111.0 (C_{3''}), 94.9 (C_{1'&3'}), 62.7 (C₄), 61.0 (C_{8'}), 58.3 (C_{10'}), 56.1 (C₃), 55.1 (C_{7'&9'}), 13.1 (C₅), -4.8 (CH₃-Si)

4-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidine-2-one (77 *cis*)

Yield of *cis* isomer isolated from mixture: 110mg (0.23 mmol) 7%

R_f: 0.66 (2:1 *n*-hexane: ethyl acetate)

¹H NMR (600 MHz, CDCl₃): δ 6.86 (d, $J_{3''-2''} = 8.3$ Hz, H_{3''}), 6.81 (dd, $J_{2''-6''} = 2.1$ Hz, $J_{2''-3''} = 8.4$ Hz, H_{2''}), 6.71 (d, 1H, $J_{6''-2''} = 2.1$ Hz, H_{6''}), 6.58 (s, 2H, H_{1'&3'}), 5.08 (d, 1H, $J_{4-3} = 5.9$ Hz, H₄), 3.82 (s, 3H, H_{10'}), 3.78 (s, 3.79, H_{8'}), 3.73 (s, 6H, H_{7'&9'}), 3.64 (dq, $J_{3-5} = 12$ Hz, $J_{3-4} = 2.6$ Hz, 1H, H₃), 1.56 (apparent bs, 3H, H₅)

¹³C NMR (100 MHz, CDCl₃): δ 168.7 (C₂), 153.3 (C_{4'&6'}), 151.4, 134.3, 133.9, 127.2, 120.6, 119.8, 112.1, 95.0, 94.8 (C_{1'&3'}), 65.9, 62.7, 61.0, 58.3, 56.1, 55.4, 49.4, 25.7, 18.4, 15.3, -4.6, -4.9

4-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-fluoro-1-(3,4,5-trimethoxyphenyl)azetidine-2-one (78)

78 was synthesised with ethyl bromofluoroacetate and **47** using **General Method VII B**.

Yield using microwave: 756 mg (1.54 mmol) 52 %

R_f: 0.56 (2:1 *n*-hexane: ethyl acetate), 0.73 (1:1 *n*-hexane: ethyl acetate)

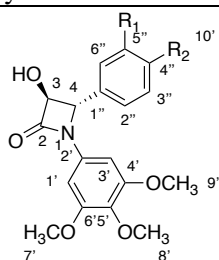
¹H NMR (400 MHz, CDCl₃): δ 6.95 (dd, 1H, $J_{2'',6''}=2.7$ Hz, $J_{2'',3''}=7.8$ Hz, H_{2''}), 6.86 (d, 1H, $J_{6'',2''}=1.7$ Hz, H_{6''}), 6.84 (d, 1H, $J_{3'',2''}=7.8$ Hz, H_{2''}), 5.83 (s, 2H, H_{1'&3'}), 5.14 (apparent dd, 1H, $^2J_{F-H3}=47.4$ Hz, $J_{3-4}=2.1$ Hz, H₃), 4.85 (apparent dd, 1H, $^3J_{F-H4}=25.3$ Hz, $J_{4-3}=2.1$ Hz, H₄), 3.81 (s, 3H, H_{10'}), 3.73 (s, 6H, H_{7'&9'}), 3.73 (s, 3H, H_{8'}), 0.97 (s, 9H, *t*-butyl), 0.10 (s, 3H, Si-CH₃), 0.09 (s, 3H, Si-CH₃)

¹³C NMR (100 MHz, CDCl₃): δ 167.9 (d, $J_{C-F}=25.6$ Hz, C₂), 153.8 (C_{4'&6'}), 150.8, 145.3, 130.7, 130.1, 120.3 (C_{2''}), 119.7 (C_{6''}), 112.2 (C_{3''}), 95.0 (C_{1'&3'}), 91.7 (C₄, $J_{F-C}=95$ Hz), 69.0, 62.0, 61.5, 61.1 (C_{8'}), 58.9 (C₃, $J_{F-C}=21$ Hz), 55.9 (C_{7'&9'}), 55.6 (C_{10'})

¹⁹F NMR (376 MHz, CDCl₃): δ -203.7

HRMS: APCI calculated for C₂₅H₃₅FNO₆Si; 492.221219, found 492.221424, error + 0.4ppm

Numbering nomenclature for 3-hydroxyl racemates



Racemate code	R ₁	R ₂
17	H	OCH ₃
18	F	OCH ₃
19	Cl	OCH ₃
20	CH ₃	OCH ₃
21	Br	OCH ₃
22	H	SCH ₃
63	NO ₂	OCH ₃
67a	OTBDMS	OCH ₃
89	H	F
90	H	NO ₂

3-Hydroxy-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (17) *trans* ³⁷⁸

17 *trans* was synthesised from **17a** using **General Method VIII**.

Overall yield for Staudinger: 900 mg (2.5 mmol) 50%

Appearance: white powder

R_f: 0.31 (2:1; *n*-hexane: ethyl acetate)

Melting Point: 122-127 °C

¹H NMR (400 MHz, CDCl₃): δ 7.26 Hz (d, 2H, $J=8.4$ Hz, H_{2''&6''}), 6.90 (d, 2H, $J=8.4$ Hz, H_{3''&5''}), 6.5 (s, 2H, H_{1'&3'}), 5.29 (s, 1H, OH), 4.79 (d, 1H, $J=1.9$ Hz, H₄), 4.74 (d, 1H, $J=1.89$ Hz, H₃), 3.80 (s, 3H, H_{10'}), 3.75 (s, 3H, H_{8'}), 3.68 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz, CDCl₃): δ 167.9 (C₂), 160.0 (C_{4'}), 153.3 (C_{4'&6'}), 135.2 (C_{5'}), 134.2 (C_{2'}), 127.8 (C_{1'}), 127.5 (C_{2'&6'}), 114.5 (C_{3'&5'}), 95.2 (C_{1'&3'}), 83.6 (C₃), 66.0 (C₄), 60.9 (C_{8'}), 55.9 (C_{7'&9'}), 55.3 (C_{10'})

IR ν_{max}(ATR): 3390 cm⁻¹ (OH), 1752 cm⁻¹ (β-lactam C=O)

LRMS: APCI calculated for [M+H⁺] 360 m/z; found 360.07

Purity (RP-HPLC): 99 %

3-Hydroxy-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (17) *Cis*

17 *cis* was synthesised from **17a** using **General Method VIII** and purified from the isomeric mixture by LC.

Overall Yield for Staudinger: 1.5%

Appearance: white powder

R_f: 0.25 (1:1; *n*-hexane:ethyl acetate)

Melting Point: 146-148 °C

¹H NMR (400 MHz, CDCl₃): δ 7.27 Hz (d, 2H, *J*=8.4 Hz, H_{2'&6'}), 6.94 (d, 2H, *J*=8.4 Hz, H_{3'&5'}), 6.58 (s, 2H, H_{1'&3'}), 5.29 (bs, 1H, OH), 5.22 (d, 1H, *J*=5.5 Hz, H₃), 5.15 (d, 1H, *J*=5.5 Hz, H₄), 3.80 (s, 3H, H_{10'}), 3.76 (s, 3H, H_{8'}), 3.71 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz, CDCl₃): δ 166.6 (C₂), 160.0, 153.5, 134.9, 133.1, 128.9, 127.9, 124.7, 114.5, 95.3, 76.7, 65.3, 62.3, 60.9, 56.0, 55.3

IR ν_{max}(ATR): 1712 cm⁻¹ (β-lactam C=O)

LRMS: APCI calculated for [M+H⁺] 360 m/z; found 360.03

Purity (RP-HPLC): 99%

4-(3-Fluoro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (18)

400

18 was obtained from **18a** using **General Method VIII**.

Overall Yield for Staudinger: 38%

Appearance: white powder

R_f: 0.32 (1:1 *n*-hexane: ethyl acetate)

Melting Point: 143-146 °C

¹H NMR (400 MHz, CDCl₃): δ 7.05 Hz (2H, apparent multiplet, H_{2'&6'}), 6.95 (t, 1H, *J*=8.5 Hz, H_{3'}), 6.47 (s, 2H, H_{7'&9'}), 4.75 (s, 1H, OH), 4.71 (d, 1H, *J*=1.3 Hz, H₄), 4.69 (d, 1H, *J*=1.3 Hz, H₃), 3.88 (s, 3H, H_{10'}), 3.75 (s, 3H, H_{8'}), 3.69 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz CDCl₃): δ 166.7 (C₂), 153.8 (C_{4'&6'}), 153.4, 151.4, 148.1, 148.0, 134.8, 132.9, 128.9, 128.8, 122.2, 113.8, 95.3 (C_{1'&3'}), 83.6 (C₄), 65.1 (C₃), 60.9 (C_{8'}), 56.3 (C_{7'&9'}), 56.0 (C_{10'})

¹⁹F NMR (376 MHz, CDCl₃): δ -133.2

IR ν_{max}(ATR): 3288 cm⁻¹ (OH), 1726 cm⁻¹ (β-lactam C=O)

LRMS: APCI calculated for [M+H⁺] 379 m/z; found 378.04

Purity (RP-HPLC): 95%

3-((*Tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (67a) ³⁷⁸

67a was obtained from **16a** using **General Method VIII**

Overall Yield for Staudinger: 24%

Melting Point: 144.5-146.9 °C

R_f: 0.38 (1:1; *n*-hexane:ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 6.89 (dd, 1H, *J*=8 Hz, *J*=2 Hz, H_{2''}), 6.82 (d, 1H, *J*=8.1 Hz, H_{3''}), 6.75 (d, 1H, *J*= 2.2 Hz, H_{6''}), 6.49 (s, 2H, H_{1'}), 4.71 (d, 1H, *J*= 1.8 Hz, H₄), 4.69 (d, 1H, *J*= 1.8 Hz, H₃), 3.78 (s, 3H, H_{10'}), 3.73 (s, 3H, H_{8'}), 3.66 (s, 6H, H_{7'&9'}), 0.9 (s, 9H), 0.05 (s, 3H), 0.05 (s, 3H)

IR ν_{max}(ATR): 3279 cm⁻¹ (OH), 1720 cm⁻¹ (β-lactam C=O)

4-(3-chloro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (19) ⁴⁰⁰

19 was obtained from **19a** using **General Method VIII**.

Overall Yield for Staudinger: 0.931g (2.37 mmol) 57%

Melting Point: 140-144 °C

R_f: 0.34 (1:1; *n*-hexane:ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 7.38 (apparent s, 1H, H_{6''}), 7.21 (d, 1H, *J*=7.5 Hz, H_{2''}), 6.95 (d, 1H, *J*=7.5 Hz, H_{3''}), 6.5 (s, 2H, H_{1'&3'}), 4.77 (apparent s, 1H, H₃), 4.75 (apparent s, 1H, H₄), 3.91 (s, 3H, H_{10'}), 3.78 (s, 3H, H_{8'}), 3.72 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz, CDCl₃): δ 166.7 (C₂), 155.4 (C_{5''}), 153.5 (C_{4'}& C_{6'}), 134.9 (C_{5'}), 132.9 (C_{2'}), 129.1 (C_{1''}), 128.1 (C_{3''}), 125.7 (C_{2''}), 123.4 (C_{1'}), 112.5 (C_{6''}), 95.4 (C_{1'&3'}), 83.1 (C₃), 65.0 (C₄), 61.0 (C_{8'}), 56.3 (C_{10'}), 56.0 (C_{7'&9'})

IR ν_{max}(ATR): 3278 cm⁻¹ (OH), 1725 cm⁻¹ (β-lactam C=O)

LRMS: APCI calculated for [M+H⁺] 391 m/z; found 391.12

Purity (RP-HPLC): 92.6%

3-hydroxy-4-(4-methoxy-3-methylphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (20)

20 was obtained from **20a** using **General Method VIII**.

Overall yield for Staudinger: 1.72 g (4.6 mmol) 46%

Melting Point: 118-122 °C

R_f: 0.20 (2:1 *n*-hexane: ethyl acetate), 0.33 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, DMSO-*d*₆): δ 7.14 (dd, 1H, *J* = 8.1 Hz, 2 Hz, H_{2''}), 7.11 (d, 1H, *J* = 1.75 Hz, H_{6''}), 6.81 (d, 1H, *J*= 7.8 Hz, H_{3''}), 6.54 (s, 2H, H_{1'&3'}), 4.77 (d, 1H, *J*= 1.8 Hz, H₄), 4.75 (d, 1H, *J* = 1.8 Hz, H₃), 3.83 (s, 3H, H_{10'}), 3.77 (s, 3H, H_{8'}), 3.71 (s, 6H, H_{7'&9'}), 2.187 (s, 3H, CH₃, H_{5''})

¹³C NMR (100 MHz, DMSO-*d*₆): δ 166.7 (C₂), 158.1 (C_{4''}), 153.4 (C_{4'&6'}), 134.6 (C_{2'}), 133.4 (C_{5'}), 128.4 (C_{3''}), 127.6 (C_{5''}), 127.4 (C_{1''}), 124.9 (C_{2''}), 110.1 (C_{6''}), 95.3 (C_{1'&3'}), 83.61 (C₃), 65.7 (C_{4'}), 60.9 (C_{8'}), 55.9 (C_{7'&9'}), 55.4 (C_{10'}), 31.0 (CH₃)

IR ν_{max}(ATR): 3287 cm⁻¹ (OH), 1727 cm⁻¹ (β-lactam C=O)

LRMS: APCI calculated for [M+H⁺] 374 m/z; found 374.02

3-hydroxy-4-(4-(methylthio)phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (22) ³⁷⁸

22 was obtained from **22a** by **General Method VIII**.

Overall yield from Staudinger: 600mg (1.6 mmol) 32%

Melting Point: 160-161 °C

R_f: 0.25 (1:1; *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, DMSO-*d*₆): δ 7.38 (d, 1H (H_{2''&6''}), 7.28 (d, 2H, *J*=8.6 Hz, H_{3''&5''}), 6.72 (d, 1H, *J*= 8.6 Hz, 3-OH), 6.52 (s, 2H, H_{1'&3'}), 4.89 (d, 1H, *J*=1.9 Hz, H₃), 4.75 (dd, 1H, *J*= 7.6 Hz, *J*= 1.9 Hz, H₄), 3.63 (s, 6H, H_{7'&9'}), 3.57 (s, 3H, H_{8'}), 2.46, (s, 3H, H_{10'})

¹³C NMR (100 MHz, DMSO-*d*₆): δ 166.9, 153.5, 138.9, 134.3, 133.8, 133.4, 127.9, 126.6, 83.8, 65.1, 60.5, 56.3, 14.9

HRMS: ESI calculated for C₁₉H₂₁NNaO₅S for [M+Na⁺] 398.103264; found 398.103717, error - 1.1 ppm

IR ν_{max}(ATR): 3300 cm⁻¹ (OH), 1721 cm⁻¹ (β-lactam C=O)

4-(3-bromo-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (21)

21 was obtained from **21a** using **General Method VIII**.

Overall yield for Staudinger: 1.48 g (3.38 mmol) 44%

Melting Point: 70 °C

R_f: 0.25 (1:1; *n*-hexane:ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 7.57 (d, 1H, *J* = 1.6 Hz, H_{6''}), 7.25 (dd, 1H, *J* = 8.3 Hz, *J* = 1.8 Hz, H_{2''}), 6.91 (d, 1H, *J* = 8.3 Hz, H_{3''}), 6.51 (s, 1H, H_{1'&3'}), 4.78 (d, 1H, *J* = 1.5 Hz, H₃), 4.74 (d, 1H, *J* = 1.5 Hz, H₄), 3.92 (s, 3H, H_{10'}), 3.78 (s, 3H, H_{8'}), 3.73 (s, 6H, H_{7'&9'})

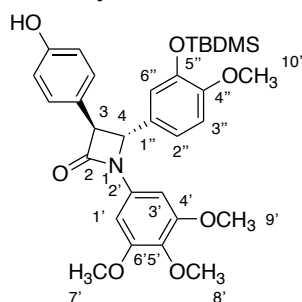
¹³C NMR (100 MHz, CDCl₃): δ 165.9 (C₂), 157.2 (C_{4''}), 153.4 (C_{4'&6'}), 134.9 (C_{5'}), 132.9 (C_{2'}), 131.2 (C_{6''}), 129.5 (C_{1''}), 126.3 (C_{2''}), 112.5 (C_{5''}), 112.3 (C_{3''}), 95.37 (C_{1'&3'}), 86.6 (C₃), 64.4 (C₄), 60.9 (C_{8'}), 56.4 (C_{7'&9'}), 56.1 (C_{10'})

IR ν_{max}(ATR): 3287 cm⁻¹ (OH), 1726 cm⁻¹ (β-lactam C=O)

LRMS: APCI calculated for [M+H⁺] 437.98 m/z; found 437.98

4-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)-3-(4-hydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (79)

79 was synthesised from **79a** using **General Method IX**.



Yield: 880mg (1.56 mmol) 55%

Appearance: white oil

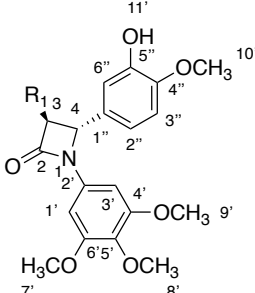
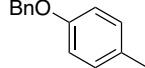
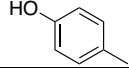
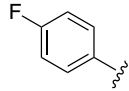
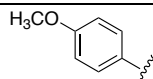
R_f: 0.65 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 7.14 (d, 2H, *J* = 8 Hz), 6.99 (d, 1H, *J* = 1.5 Hz, H_{6''}), 6.83 (dd, 1H, *J* = 1.5 Hz, *J* = 6 Hz, H_{2''}), 6.80 (d, 1H, *J* = 8 Hz, H_{3''}), 6.77 (d, 2H, *J* = 8 Hz), 6.64 (s, 2H, H_{1'&3'}), 4.72 (d, 1H, *J* = 1.9 Hz, H₃), 4.16 (d, 1H, *J* = 1.9 Hz, H₄), 3.79 (3H, s, H_{10'}), 3.75 (s, 3H, H_{8'}), 3.65 (s, 6H, H_{7'&9'}), 0.91 (s, 9H, C(CH₃)₂), 0.07 (s, 3H, Si-CH₃), 0.04 (s, 3H, Si-CH₃)

¹³C NMR (100 MHz, CDCl₃): δ 166.3, 153.5, 133.6, 130.5, 129.7, 128.7, 119.5, 118.4, 115.9, 115.5, 112.5, 94.9, 64.3, 64.2, 61.13, 60.9, 55.9, 55.5

IR ν_{max}(ATR): 3299.01 cm⁻¹ (OH), 1737 cm⁻¹ (β-lactam C=O)

Numbering nomenclature for B ring *meta* hydroxyl substituted racemates

	
Code	R ₁
16	OH
67b	
24	
83	
26	
27	H
28	CH ₃
29	F
30	
33	
39	
84	CBZ
41	

3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (16)

378

16 was synthesised from both **67a** using **General Method X**.

Overall yield for Staudinger from 67a: 1.2g (2.6 mmol) 43%

Overall Yield for Staudinger from 67b: 56 mg (0.15 mmol) 67 %

R_f: 0.2 (1:1; *n*-hexane:ethyl acetate)

Melting Point: 62-64 °C

¹H NMR (400 MHz, CDCl₃): δ 7.29 (singlet, 1H, OH), 6.65 (apparent singlet, 1H, H_{6''}), 6.85 (apparent singlet, 2H, H_{2''&3''}), 6.53 (s, 2H, H_{1'&3'}), 4.75 (1H, apparent singlet, H₃), 4.73 (1H, apparent singlet, H₄), 3.90 (s, 3H, H_{10'}), 3.77 (s, 3H, H_{8'}), 3.71 (s, 6H, (H_{7'&9'}))

¹³C NMR (100 MHz, CDCl₃): δ 167.0, 153.8 (C_{4'&6'}), 147.1 (C_{4''}), 147.0 (C_{5''}), 135.0 (C_{5'}), 133.3 (C_{2'}), 130.0 (C_{1''}), 118.2 (C_{2''}), 112.2 (C_{3''}), 111.0 (C_{6''}), 95.5 (C_{1'&3'}), 83.8 (C₄), 65.7 (C₃), 61.0 (C_{8'}), 56.0 (C_{7'&9'}), 56.0 (C_{10'}), 54.0 (C_{8'})

IR ν_{max} (ATR): 3357 cm⁻¹ (OH), 1747 cm⁻¹ (β-lactam C=O)

LRMS: APCI calculated for [M+H⁺] 376 m/z; found 376.04

Purity (HPLC): 95%

2-(3-hydroxy-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl acetate (67b)

67b was synthesised from **16a** using **General Method X**.

Overall Yield for Staudinger: 680 mg (1.63 mmol) 32%

R_f: 0.4 (1:1; *n*-hexane:ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 6.02 (d, 1H, *J* = 1.6 Hz, H_{6''}), 6.88 (m, 2H, H_{2'''&3'''}), 6.54 (s, 2H, H_{1'&3'}), 5.38 (d, *J* = 1.5 Hz, 1H, H₃), 4.80 (d, *J* = 1.5 Hz, 1H, H₄), 3.89 (s, 3H, H_{10'}), 3.76 (s, 3H, H_{8'}), 3.70 (s, 6H, H_{7'&9'}), 2.17 (s, 3H, OCH₃)

¹³C NMR (100 MHz, CDCl₃): δ 169.6 (C=O, OCH₃), 161.6 (C₂), 153.5 (C_{4'&6'}), 147.3 (C_{4''}), 146.3 (C_{5''}), 134.2 (C_{2'}), 133.1 (C_{5'}), 128.5 (C_{1''}), 118.5 (C_{2''}), 112.7 (C_{6''}), 111.1 (C_{3''}), 95.5 (C_{1'&3'}), 82.3 (C₂), 63.5 (C₁), 60.9 (C_{8'}), 56.2 (C_{10'}), 55.9 (C_{7'&9'}), 20.7 (CH₃, OCH₃)

4-(3-Hydroxy-4-methoxyphenyl)-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (24)

378

24 was synthesised from **70** using **General Method X**.

Overall yield for Staudinger: 766 mg (1.76 mmol) 17.6 %

R_f: 0.4-0.5 (2:1 *n*-hexane: ethyl acetate)

Melting Point: 81-85 °C

¹H NMR (400 MHz, CDCl₃): δ 7.35 (m, 5H, 3-phenyl), 6.99 (d, 1H, *J* = 2 Hz, H_{6''}), 6.91 (dd, 1H, H_{2'}, *J* = 8 Hz, *J* = 2 Hz, H_{2''}), 6.86 (d, 1H, *J* = 8 Hz, H_{3''}), 6.64 (s, 2H, H_{1'&3'}), 5.79 (s, OH), 4.80 (d, 1H, *J* = 2.5 Hz, H₃), 4.29 (d, 1H, *J* = 2.5 Hz, H₄), 3.90 (s, 3H, H_{10'}), 3.76 (s, 3H, H_{8'}), 3.72 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz, CDCl₃): δ 165.5 (C₂), 153.4 (C_{4'&6'}), 146.8 (C_{4''}), 146.3 (C_{5''}), 134.7 (C_{5'}), 133.7 (C_{2'}), 129.0 (C_{1''}), 127.87, 127.4, 117.7 (C_{2''}), 112.0 (C_{3''}), 110.9 (C_{6''}), 94.9 (C_{1'&3'}), 64.9 (C₃), 63.7 (C₄), 61.0 (C_{8'}), 56.0 (C_{7'&9'}), 55.9 (C_{10'})

IR ν_{max}(ATR): 3183 cm⁻¹ (OH), 1775 cm⁻¹ (β-lactam C=O)

LRMS: APCI calculated for [M+H⁺] 436 m/z; found 436.13

3-(4-(benzyloxy)phenyl)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (83)

83 was synthesised from **79a** using **General Method X**.

Yield: not calculated

Appearance: yellow oil/gum

R_f: 0.67 (1:1 *n*-hexane:ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 7.45 – 7.33 (m, 5H, Bn Ar-H), 7.25 (d, 2H, *J* = 8.8 Hz, H_{2''&7''}), 7.22 (dd, 1H, *J* = 7.0 Hz, *J* = 2.2 Hz, H_{3''}), 7.16 (d, 1H, *J* = 2.2 Hz, H_{6'}), 6.99 (d, 3H, *J* = 8.8 Hz, H_{3'''&5'''}, H_{3''}), 6.63 (2H, H_{1'&3'}), 5.09 (s, 2H, benzyl CH₂), 4.80 (d, 1H, *J* = 2.6 Hz, H₃), 2.47 (d, 1H, *J* = 2.6 Hz, H₄), 3.87 (s, 3H, H_{10'}), 3.79 (s, 3H, H_{8'}), 3.76 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz, CDCl₃): δ 166.2, 158.5, 153.3, 153.2, 146.8, 146.2, 136.7, 133.9, 130.7, 128.6, 127.6, 127.1, 117.8, 115.6, 112.1, 111.1, 97.5, 94.7, 70.1, 64.5, 64.1, 60.9, 56.2

4-(3-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (26)

26 was synthesised from **79b** using **General Method X**.

Appendices

Overall yield for Staudinger: 880 mg (1.56 mmol) 11%

Appearance: white powder

R_f: 0.24 (1:1 *n*-hexane: ethyl acetate)

Melting Point: 73-75 °C

¹H NMR (400 MHz, CDCl₃): δ 7.13 (d, 2H, *J*=8 Hz), 6.98 (d, 1H, *J*=1.5 Hz, H_{6''}), 6.91 (dd, 1H, *J*=8 Hz, *J*=1.5 Hz, H_{5''}), 6.87 (d, 1H, *J*=8 Hz, H_{3''}), 6.78 (d, 2H, *J*=8 Hz), 6.64 (s, 2H, H_{1'&3'}), 4.78 (d, 1H, *J*=1.9 Hz, H₃), 4.19 (d, 1H, *J*=1.9 Hz, H₄), 3.92 (3H, s, H_{10'}), 3.80 (s, 3H, H_{8'}), 3.75 (s, 6H, H_{7'&9'})

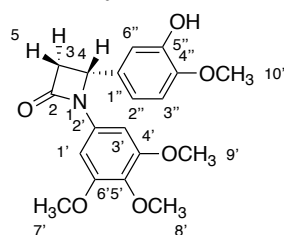
¹³C NMR (100 MHz, CDCl₃): δ 166.7 (C₂), 155.7 (C_{5''}), 153.4 (C_{4'&6'}), 147.0 (C_{4''}), 146.3 (C_{5'}), 133.6 (C_{2'}), 130.6 (C_{1''}), 128.5 (C₄), 126.1, 117.9 (C_{3''}), 116.03, 111.9 (C_{6''}), 111.1 (C_{2'}), 94.9 (C_{1'&3'}), 66.0 (C₃), 64.2 (C₄), 61.1 (C_{8'}), 56.1 (C_{7'&9'}), 56.0 (C_{10'})

IR ν_{max}(ATR): 1725.88 cm⁻¹ (β-lactam C=O)

LRMS: APCI calculated for [M+H⁺] 452 m/z; found 451.10

4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (27)

27 was synthesised from **76** using **General Method X**.



Yield (microwave): 110 mg (0.31 mmol) 5%

Yield (reflux): 900 mg (2.5 mmol) 36 %

R_f: 0.25 (1:1 *n*-hexane: ethyl acetate)

Melting Point: 103-104°C

¹H NMR (400 MHz, CDCl₃): δ 6.98 (d, 1H, ³*J*_{6''-2''}=1.8 Hz, H_{6''}), 6.91 (dd, 1H, ³*J*_{2''-3''}=8.4 Hz, ³*J*_{2''-6''}=1.3 Hz, H_{2''}), 6.86 (d, 1H, ³*J*_{3''-2''}=8.3 Hz, H_{3''}), 6.58 (s, 2H, H_{1'&3'}), 5.7 (bs, 1H, H₈, OH), 4.89 (dd, 1H, ³*J*₄₋₅=5.5 Hz, ³*J*₄₋₃=2.7 Hz, H₄), 3.92 (s, 3H, H_{10'}), 3.78 (s, 3H, H_{8'}), 3.75 (s, 6H, H_{7'&9'}), 3.53 (dd, 1H, ²*J*₅₋₃=13.7 Hz, ³*J*₅₋₄=5.1 Hz, H₅), 2.95 (dd, 1H, ³*J*₃₋₅=14.6 Hz, *J*₃₋₅=2.7 Hz, H₃)

¹³C NMR (100 MHz, CDCl₃): δ 165.0, 153.5, 146.1, 117.7, 112.0, 110.9, 94.6, 77.3, 76.7, 60.9, 56.1, 54.1, 46.9

IR ν_{max}(ATR): 1732.58 cm⁻¹ (β-lactam C=O)

LRMS: APCI calculated for [M+H⁺] 360 m/z; found 360.11

(4-(3-hydroxy-4-methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (28 *cis*))

28 *cis* was synthesised from **77 *cis*** using **General Method X**.

Yield for Reformatsky: 50 mg (0.13 mmol) 2.6%

Appearance: yellow powder

R_f: 0.3 (1:1 *n*-hexane: ethyl acetate)

Melting Point: 144-145 °C

¹H NMR (400 MHz, CDCl₃): δ 6.83 (d, 1H, ³*J*_{3''-2''}=8.2 Hz, H_{3''}), 6.81 (d, 1H, *J*_{6''-2''}=1.7 Hz, H_{6''}), 6.72 (dd, 1H, *J*_{2''-3''}=8.2 Hz, *J*_{2''-6''}=1.7 Hz, H_{2''}), 6.57 (s, 2H, H_{1'&3'}), 5.71 (bs, 1H, OH),

5.06 (d, 1H, J_{3-4} = 6.1 Hz, H₃), 3.84 (s, 3H, H_{10'}), 3.77 (s, 3H, H_{8'}), 3.72 (s, 6H, H_{7'&9'}), 3.62 (dq, 1H, J_{3-4} = 6.1 Hz, J_{3-5} = 6.7 Hz, H₃), 0.91 (d, 3H, J_{5-3} = 6.92 Hz, H₅)

¹³C NMR (100 MHz, CDCl₃): δ 168.6, 153.5, 146.5, 145.8, 134.3, 134.0, 128.1, 119.3, 118.6, 113.8, 113.3, 111.3, 110.8, 95.5, 95.2, 94.6, 57.9, 56.2, 49.4, 49.2

IR ν_{max}(ATR): 3196.7 cm⁻¹ (OH, intermolecular bonded), 2930.4 cm⁻¹ (OH, intramolecular bonded) 1708 cm⁻¹ (β-lactam C=O)

HRMS: APCI calculated for C₂₀H₂₄NO₆ [M + H⁺] 374.159814; 374.159684, error + 0.3 ppm

(4-(3-hydroxy-4-methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (28 *trans*)

28 *trans* was synthesised from **77** *trans* using **General Method X**.

Yield (reflux) for desilylation and purification: 40 mg (0.11 mmol) 61%. **Yield for**

Reformatsky: 140mg (0.38 mmol) 7.6%

Appearance: pale yellow powder

R_f: 0.35 (1:1 *n*-hexane: ethyl acetate), 0.5 (1:2 *n*-hexane: ethyl acetate)

Melting Point: 140-142 °C

¹H NMR (600 MHz, DMSO-*d*₆): δ 6.92 (d, 1H, $J_{3''-2''}$ = 8.5 Hz, H_{3''}), 6.81 (dd, 1H, $J_{2''-3''}$ = 8.9 Hz, $J_{2''-6''}$ = 1.7 Hz, H_{2''}), 6.82 (d, 1H, $J_{6''-2''}$ = 1.7 Hz, H_{6''}), 6.55 (s, OH), 6.53 (s, 2H, H_{1'&3'}), 4.69 (d, 1H, J_{4-3} = 2.5 Hz, H₄), 3.71 (s, 3H, H_{10'}), 3.63 (s, 6H, H_{7'&9'}), 3.59 (s, 3H, H_{8'}), 3.10 (dq, 1H, J_{3-4} = 2.5 Hz, J_{4-5} = 8 Hz, H₃), 1.34 (d, 3H, J_{5-3} = 6.92 Hz, H₅)

¹H NMR (400 MHz, CDCl₃): δ 6.96 (d, 1H, J = 1.8 Hz, H_{6''}), 6.87 (m, 3H, H_{2''&3''}), 6.58 (s, 2H, H_{1'&3'}), 5.70 (s, 1H, OH), 4.46 (d, 1H, J = 2 Hz, H₄), 3.92 (s, 3H, H_{10'}), 3.78 (s, 3H, H_{8'}), 3.75 (s, H_{7'&9'}), 3.13 (dq, 1H, J = 8.9 Hz, J = 2 Hz, H₃), 1.47 (d, 3H, J = 6.4 Hz)

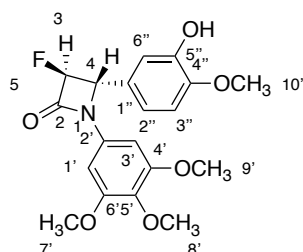
¹³C NMR (100 MHz, DMSO-*d*₆): δ 167.8 (C₂), 153.0 (C_{4'&6'}), 147.7 (C_{4''}), 146.9 (C_{5''}), 133.6 (C_{5'}), 130.3 (C_{1''}), 117.6 (C_{2''}), 113.1 (C_{6''}), 112.2 (C_{3''}), 94.6 (C_{1'&3'}), 61.2 (C₄), 56.1 (C_{7'&9'}), 55.6 (C_{10'}), 54.8 (C₄), 53.9 (C₈), 12.8 (CH₃)

IR ν_{max}(ATR): 2930 cm⁻¹ (OH), 1732 cm⁻¹ (β-lactam C=O)

HRMS: APCI calculated for C₂₀H₂₄NO₆ [M⁺ + H], 374.159814; 374.159938, error + 0.3 ppm.

3-fluoro-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (29)

29 was synthesised from **78** using **General Method X**.



Yield for Reformatsky: 640 mg (1.7 mmol) 56%

Appearance: Yellow powder

R_f: 0.25 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 6.98 (d, 1H, J = 1.9 Hz, H_{6''}), 6.88 (dd, 1H, J = 8.1 Hz, J = 1.9 Hz, H_{2''}), 6.83 (d, 1H, J = 8.9 Hz, H_{3''}), 5.83 (s, 2H, H_{1'&3'}), 5.11 (apparent dd, 1H, $^2J_{F-3}$ = 48.3 Hz, $^3J_{3-4}$ = 2.6 Hz, H₃), 4.86 (apparent dd, 1H, $^3J_{F-5}$ = 26.1 Hz, J_{4-3} = 2.6 Hz, H₄), 3.87 (s, 3H, H_{10'}), 3.74 (s, 6H, H_{7'&9'}), 3.72 (s, 3H, H_{8'}),

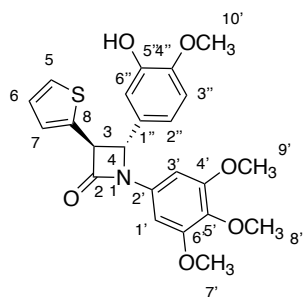
¹³C NMR (100 MHz, CDCl₃): δ 167.91 (*J*_{F-C} = 31 Hz, C₂), 153.8 (C_{4'&6'}), 146.2 (*J*_{F-C} = 62 Hz, C_{2'}), 142.8, 131.4 (C₂), 130.4 (C_{5'}), 118.6 (C_{2''}), 113.1 (C_{6''}), 110.8 (C_{3''}), 95.2 (*J*_{F-C} = 75 Hz, C₃), 92.3 (C_{1'&3'}), 62.1 (C_{8'}), 61.0, 58.8 (*J*_{F-C} = 26 Hz, C₄), 56.0 (C_{8'}) 55.9 (C_{7''&9})

¹⁹F NMR (376 MHz, CDCl₃): δ -203.74

HRMS: APCI calculated for C₁₉H₂₁FNO₆ [M + H⁺], 378.1353; found 378.1347.

4-(3-hydroxy-4-methoxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (30)

30 was synthesised from **71** using **General Method X**.



Overall yield for Staudinger: 870 mg (1.61 mmol) 32%

Appearance: yellow powder

Melting point: 118-120 °C

R_f: 0.29 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 7.31 (dd, 1H, *J* = 7 Hz, *J* = 1.7 Hz), 7.10 (d, 1H, *J* = 1.8 Hz), 7.05 (dd, 1H, *J* = 8.41 Hz, *J* = 3.5 Hz, H₆), 7.01 (d, 1H, *J* = 2.1 Hz), 6.94 (dd, 1H, *J* = 8.14 Hz, *J* = 1.9 Hz), 6.89 (d, 1H, *J* = 8.9 Hz, H_{3''}), 6.61 (s, 2H, H_{1'&3'}), 5.70 (bs, 1H, OH), 4.87 (d, 1H, *J* = 1.8 Hz, H₃), 4.48 (d, 1H, *J* = 1.8 Hz, H₄), 3.93 (s, 3H, H_{10'}), 3.80 (s, 3H, H_{8'}), 3.76 (s, 6H, H_{7'&9'})

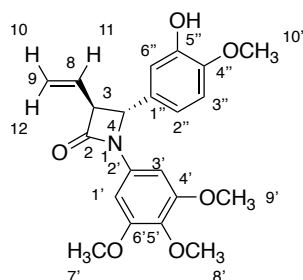
¹³C NMR (100 MHz, CDCl₃): δ 164.4 (C₂), 153.6 (C_{4'&6'}), 146.9 (C_{4''}), 146.4 (C_{5''}), 136.1, 134.7, 133.6, 129.9, 127.3, 125.8, 125.3, 117.7, 112.0, 111.0, 94.9, 64.5, 60.9, 60.1, 56.1

IR ν_{max} (ATR): 3528 cm⁻¹ (free OH), 3187 cm⁻¹ (intramolecular bonded OH), 1719 cm⁻¹ (β-lactam C=O)

LRMS: APCI calculated for [M+H⁺] 442 m/z; found 442.09

4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-one (33)

33 was synthesised from **72** using **General Method X**.



Yield: 530 mg (1.38 mmol) 28%

Appearance: white powder

R_f: 0.35 (1:1 *n*-hexane: ethyl acetate)

Melting Point: 58-60 °C

¹H NMR (400 MHz, CDCl₃): δ 6.97 (d, 1H, H_{6''}, ³J_{6''-2''} = 2.1 Hz), 6.88 (dd, 1H, ³J_{2''-3''} = 8Hz, J_{2''-6''} = 2.1 Hz, H_{2''}), 6.87 (d, 1H ³J_{3''-2''} = 8Hz, H_{3''}), 6.58 (s, 2H, H_{1'&3'}), 6.03 (td, 1H, J₁₁₋₁₂ = 17.1 Hz, J₁₁₋₁₀ = 9.9 Hz, J₁₁₋₃ = 2.2 Hz, H₁₁), 5.71 (bs, OH), 5.40 (dd, 1H, ³J₁₂₋₁₁ = 17 Hz, ²J₁₂₋₁₀ (geminal) = 2.2 Hz, H₁₂), 5.33 (dd, 1H, ³J₁₀₋₁₁ = 9.9 Hz, ²J₁₀₋₁₂ (geminal) = 2.2 Hz, H₁₀), 4.69 (d, 1H, J₄₋₃ = 2.2 Hz, H₄), 3.92 (s, 3H, H_{8'}), 3.78 (s, 3H, H_{10'}), 3.75 (s, 6H, H_{7'&9'}), 3.74 (dt, 1H, ³J₃₋₁₁ = 8.5 Hz, ³J₃₋₄ = 2.2 Hz, H₃)

¹³C NMR (100 MHz, CDCl₃): δ 165.3 (C₂), 153.5 (C_{1'&3'}), 146.9 (C_{4''}), 146.3 (C_{5''}), 135.2 (C_{5'}), 133.9 (C_{2'}), 130.6 (C_{1''}), 130.5 (C₉), 119.8 (C₈), 117.8 (C_{2''}), 112.0 (C_{6''}), 111.9 (C_{3''}), 94.8 (C_{1'&3'}), 63.9 (C₃), 61.4 (C₄), 60.9 (C_{8'}), 56.1 (C_{7'&9'}), 56.0 (C_{10'})

IR ν_{max}(ATR): 1738 cm⁻¹ (β-lactam C=O)

4-(3-hydroxy-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (38)

38 was synthesised from 73 using General Method X.

Yield from Staudinger: 700 mg (1.57 mmol) 32%

Appearance: white powder

Melting Point: 61-64 °C (amorphous powder)

R_f: 0.15 (1:1 *n*-hexane: ethyl acetate)

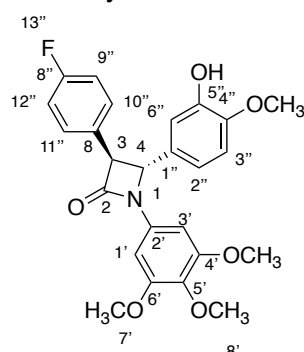
¹H NMR (400 MHz, CDCl₃): δ 7.28 (d, 2H, *J* = 8.9 Hz), 7.03 (t, 1H, *J* = 8.9), 7.0 (d, 1H, *J* = 3.2 Hz), 6.96 (dd, 1H, *J* = 7.9 Hz, *J* = 3.2 Hz), 6.92 (d, 1H, *J* = 7.9 Hz), 6.90 (d, 2H, *J* = 8.9 Hz), 6.61 (s, 2H), 5.74 (s, 1H, OH), 5.13 (d, 1H, *J* = 1.3 Hz), 4.90 (d, 1H, *J* = 1.3 Hz), 3.96 (s, 3H), 3.79 (s, 3H), 3.74 (s, 6H)

¹³C NMR (100 MHz, CDCl₃): δ 162.5, 157.1, 153.6, 147.3, 146.6, 134.9, 133.1, 129.7, 122.2, 118.5, 115.9, 115.4, 112.4, 111.1, 95.4, 87.1, 64.0, 60.8, 55.9

IR ν_{max}(ATR): 3386 cm⁻¹ (OH), 1749 cm⁻¹ (β-lactam C=O)

3-(4-fluorophenyl)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (39)

39 was synthesised from 73 using General Method X.



Yield: 900mg (1.98 mmol) 40 %

Appearance: yellow fluffy powder

Melting Point: 99-101 °C

R_f: 0.35 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 7.33 (apparent dd, 2H, *J* = 8.1 Hz, *J* = 3.1 Hz, H_{9''&12''}), 7.09 (apparent triplet, 2H, *J* = 8.1 Hz, H_{10''&11''}), 7.00 (d, 1H, *J* = 2.1 Hz, H_{6''}), 6.93 (dd, 1H, *J* = 7.9, *J* = 1.8 Hz, H_{2''}), 6.89 (d, 1H, *J* = 7.9, H_{3''}), 6.63 (s, 2H, H_{1'&3'}), 5.72 (s, 1H, OH), 4.78 (d, 1H, *J* = 1.8 Hz, H₃), 4.27 (d, 1H, *J* = 1.8 Hz, H₄), 3.94 (s, 3H, H_{10'}), 3.80 (s, 3H, H_{8'}), 3.76 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz, CDCl₃): δ 161.3 (C₂), 153.6 (C_{1'&3'}), 147.0, 146.4, 130.3, 129.0, 117.8, 116.2, 115.9, 115.3, 112.0, 111.2, 94.9, 85.1 (C₃), 64.3 (C₄), 64.0, 64.0, 61.1, 56.2 (C_{7'&9'})

¹⁹F NMR (376 MHz, CDCl₃): δ -114.1

IR ν_{max} (ATR): 1749.79 cm⁻¹ (β-lactam C=O)

LRMS: APCI calculated for [M+H⁺] 454 m/z; found 454.15

Benzyl (4-(2-(3-hydroxy-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3-yl)phenyl) carbamate (84)

84 was synthesised from **43** using **General Method X**.

Yield: 90 mg (0.15 mmol) 11.1%

Appearance: yellow powder

Melting Point: 95-97 °C

R_f: 0.45 (1:2 *n*-hexane: ethyl acetate)

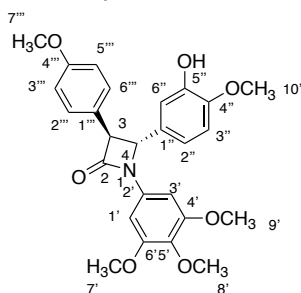
¹H NMR (400 MHz, CDCl₃): δ 7.42-7.32 (m, 7H), 7.27 (d, 2H, *J* = 8.6 Hz), 6.99 (d, 1H, *J* = 1.8 Hz, H_{6''}), 6.9 (dd, 1H, *J* = 8.6 Hz, *J* = 1.8 Hz, H_{2''}), 6.88 (d, 1H, *J* = 8.6 Hz, H_{3''}), 6.81 (bs, 1H, NH), 6.63 (s, 2H, H_{1'&3'}), 5.22 (s, 2H, CH₂ of CBZ group), 4.77 (d, 1H, *J* = 2.2 Hz, H₃), 4.24 (d, 1H, *J* = 2.2 Hz, H₄), 3.92 (s, 3H, H_{10'}), 3.8 (s, 3H, H_{8'}), 3.75 (s, 6H, H_{7'&9'}),

¹³C NMR (100 MHz, CDCl₃): δ 165.6, 153.5, 146.9, 146.5, 137.5, 134.6, 133.7, 130.4, 128.7, 128.3, 119.1, 117.8, 111.9, 111.0, 95.1, 67.1, 64.5, 63.4, 61.0, 56.0

IR ν_{max} (ATR): 2931 cm⁻¹ (NH), 1727 cm⁻¹ (β-lactam C=O)

4-(3-hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (41)

41 was synthesised from **75** using **General Method X**.



Yield for Staudinger: 1.2g (2.58 mmol) 43%

Appearance: yellow powder

Melting Point: 93 °C

R_f: 0.35 (:1 *n*-hexane: ethyl acetate)

Melting Point: 81 °C

¹H NMR (400 MHz CDCl₃): δ 7.25 (d, 2H, *J* = 8.2 Hz, H_{2'''&6'''}), 7.0 (d, 1H *J* = 2 Hz, H_{6''}), 6.92 (d, 2H, *J* = 8.2 Hz, H_{3'''&5'''}), 6.92 (dd, *J* = 8.1 Hz, *J* = 2 Hz, H_{2''}), 6.9 (d, *J*_{3''-2''} = 8.1 Hz, H_{3''}), 6.63 (s, 2H, H_{1'&3'}), 5.75 (broad singlet, OH), 4.77 (d, 1H, *J* = 2.1 Hz, H₃), 4.23 (d, 1H, *J* = 2.1 Hz, H₄), 3.92 (s, 3H, H_{10'}), 3.83 (s, 3H, H_{7'''}), 3.79 (s, 3H, H_{8'}), 3.75 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz CDCl₃): δ 166.0 (C₂), 159.3 (C_{4'''}), 153.5 (C_{4'&6'}), 146.8, 146.4, 134.5 (C_{5'}), 133.8 (C_{2'}), 130.7, 128.6 (C_{6''}), 126.9 (C_{2''}), 117.8 (C_{6''}), 114.4, 112.1 (C_{3''}), 111.0, 94.9 (C_{1&3}), 64.5 (C₄), 64.2 (C₃), 56.1 (C_{7'&9'}), 56.0 (C_{10'}), 55.3 (C_{7'''})

IR ν_{max} (ATR): 2937 cm⁻¹ (OH), 1736 cm⁻¹ (C=O)

LRMS: APCI calculated for [M+H⁺] 466 m/z; found 466.19

4-(3-amino-4-methoxyphenyl)-3-phenoxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (37 *trans*)

37 trans was synthesised from **36 trans** using **General Method XI**.

Yield for reduction and purification: 160 mg (0.36 mmol) 38%

Overall yield for Staudinger: 7%

Appearance: brown oil/powder

R_f: 0.32 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): 7.28 (m, 2H), 7.01 (t, 2H, *J* = 7.0 Hz), 6.95 – 6.85 (m, 4H), 6.62 (s, 2H), 5.13 (d, 1H, *J* = 1.9 Hz), 4.88 (d, 1H, *J* = 1.9 Hz), 3.91 (s, 3H), 3.79 (s, 3H), 3.74 (s, 6H)

¹³C (100 MHz, CDCl₃): not determined as yield was too low.

4-(3-amino-4-methoxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (32 as an isomeric mixture)

32 was synthesised from **31** as a mixture of *cis:trans* isomers (ratio 32: 68) prior to further purification as *cis* and *trans* isomers using **General Method XI**.

Yield for reduction and purification: 410 mg (0.93 mmol) 33%

Yield for Staudinger: 11%

Appearance: yellow powder

Melting Point: 65-69 °C (amorphous powder)

R_f: 0.31 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (600 MHz, CDCl₃): δ 7.29 (dd, 1H, *J* = 4.5 Hz, *J* = 1.3 Hz, thiophene), 7.28 (m, 2H, H_{5&6}), 7.24 (bs, 1H, NH₂), 7.1 – 7.05 (m, 3H, thiophene ring), 7.03 (d, 1H, *J* = 3.3 Hz, thiophene ring), 6.91 (d, 0.4H, *cis* isomer, *J* = 3.3 Hz, H_{6''}), 6.83 (m, 1H), 6.64 (s, 2H, H_{1'&3'}), 6.58 (d, 0.4H, *cis* isomer, *J* = 9 Hz, H_{3''}), 5.30 (d, 0.4H, *cis* isomer, *J* = 4.6 Hz, H₃), 5.08 (d, 0.4H, *cis* isomer, *J* = 4.6 Hz, H₄), 4.83 (d, 1H, *J* = 3.3 Hz, H₃), 4.48 (d, 1H, *J* = 3.3 Hz, H₄), 3.96 (s, 1.8H, *cis* isomer, H₁₀), 3.89 (s, 3H, H_{10'}), 3.82 (s, 3H, H_{8'}), 3.79 (s, 2.5H, *cis* isomer, H_{7'&9'}), 3.76 (6H, H_{1'&3'})

¹³C NMR (100 MHz, CDCl₃): δ 164.5, 153.6, 136.2, 134.7, 133.8, 129.6, 127.8, 126.6, 125.3, 110.8, 95.0 (C_{1'&3'}), 65.0 (C_{3 trans}), 61.13 (C_{8'}), 60.8, 60.0, 56.3, 55.9 (C_{7'&9'}), 55.6 (C_{10'})

¹H NMR (400 MHz, DMSO-*d*₆): δ 7.56 (dd, 1H, *J* = 5.2 Hz, *J* = 1.2 Hz), 7.15 (d, 1H, *J* = 3.7 Hz), 7.09 (dt, 1H, *J* = 5.3, *J* = 1.5 Hz), 6.83 (d, 1H, *J* = 8.5 Hz, H_{3''}), 6.77 (d, 1H, *J* = 1.5 Hz, H_{6''}), 6.75 (dd, 1H, *J* = 9.1 Hz, *J* = 1.5 Hz, H_{2''}), 6.63 (s, 2H, H_{1'&3'}), 5.07 (d, 1H, *J* = 2.2 Hz, H₃), 4.87 (bs, 1H, OH), 4.64 (d, 1H, *J* = 2.2 Hz, H₄), 3.79 (s, 3H, OCH₃), 3.68 (s, 6H, H_{1'&3'}), 3.61 (s, 3H, OCH₃)

¹³C NMR (100 MHz, DMSO-*d*₆): δ 164.8 (C₂), 153.6, 147.2, 138.8, 136.9, 134.5, 133.7, 129.5, 127.8, 126.6, 126.4, 115.4, 111.0, 97.3, 95.6, 64.2, 60.6, 56.3, 55.8

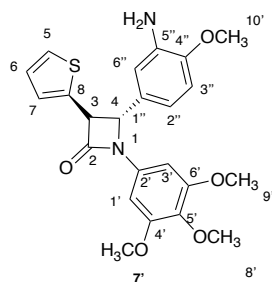
¹⁵N NMR (60.8 MHz, CDCl₃): δ 131.2 (NH₂)

IR ν_{max}(ATR): 1745 cm⁻¹ (β-lactam C=O)

HRMS: APCI calculated for C₂₃H₂₅N₂O₅S [**M + H**⁺], 441.147869; found 441.147664, error + 0.5 ppm

4-(3-amino-4-methoxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (32 *trans*)

32 trans was isolated from a mixture of *cis:trans* isomers using LC



Yield: 240 mg (0.55 mmol) 59 % of isomeric composition

Appearance: yellow powder

R_f: 0.35 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (600 MHz, CDCl₃): δ 7.30 (dd, 1H, *J* = 4.5 Hz, *J* = 1.3 Hz, H₆), 7.2 (bs, 2H, NH₂), 7.09 (apparent d, 1H, *J* = 2.6 Hz, H₇), 7.05 (dd, 1H, *J* = 4.5 Hz, *J* = 2.1 Hz, H₅), 6.93 (m, 2H, H_{1'}&3'), 6.85 (d, 1H, *J* = 8.5 Hz, H_{3''}), 6.63 (s, 2H, H_{1'}&3'), 4.85 (d, 1H, *J* = 1.8 Hz, H₃), 4.48 (d, 1H, *J* = 1.8 Hz, H₄), 3.89 (s, 3H, H_{10'}), 3.79 (s, 3H, H_{8'}), 3.76 (s, 3H, H_{7'}&9')

¹³C NMR (100 MHz, CDCl₃): δ 164.4, 153.6, 136.2, 134.6, 133.7, 129.5, 127.3, 125.8, 125.3, 111.0, 95.1, 64.7, 61.0, 60.2, 56.2, 55.9

¹⁵N NMR (60.8 MHz, CDCl₃): δ 130.8

IR ν_{max}(ATR): 1745 cm⁻¹ (β-lactam C=O)

HRMS: APCI calculated for C₂₃H₂₅N₂O₅S [**M+H**⁺], 441.147869; found 441.147664, error + 0.5 ppm

4-(3-amino-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-one (35)

35 was synthesised from **34** using **General Method XI**

Yield for reduction and purification: 400mg (1.04 mmol) 90 %

Yield for Staudinger: 20%

Appearance: off white amorphous powder

R_f: 0.41 (1:1 *n*-hexane: ethyl acetate)

Melting Point: 60-65 °C

¹H NMR (400 MHz, CDCl₃): δ 6.78 (apparent doublet, *J* = 6.6 Hz, 3H, H_{2''}, 3''&6''), 6.6 (s, 2H, H_{1'}&3'), 6.01 (td, 1H, ³*J*_{13'-11'} = 17.1 Hz, ³*J*_{13'-12'} = 9.9 Hz, ³*J*_{13'-3} = 2.2 Hz, H_{13'}), 5.38 (d, 1H, ³*J*_{11'-13'} = 16 Hz, H_{11'}), 5.32 (d, 1H, ³*J*_{12'-13'} = 9.9 Hz, H_{12'}), 5.32 (d, 1H, *J*₄₋₃ = 2.2 Hz, H₄), 3.88 (s, 3H, H_{10'}), 3.78 (s, 3H, H_{8'}), 3.75 (apparent singlet, 7H, H_{7'}&9' & H₄)

¹³C NMR (100 MHz, CDCl₃): δ 165.7, 153.9, 134.3, 133.9, 130.9, 129.9, 119.7, 110.7, 94.9, 63.8, 60.9, 56.2, 55.7, 30.9

IR ν_{max}(ATR): 1736 cm⁻¹ (β-lactam C=O)

4-(3-Amino-4-methoxyphenyl)-3-(methylperoxy)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (23a)

23a was synthesised from **63a** using General Method XII.

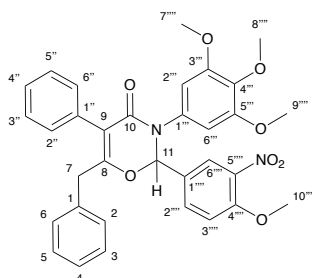
Yield: <15%

R_f: 0.25 (1:4 *n*-hexane: ethyl acetate)

¹H NMR (400 MHz, CDCl₃): δ 8.55 (d, 1H, *J* = 1.8 Hz, H_{6''}), 7.81 (bs, 1H, NH₂), 7.02 (dd, 1H, *J* = 1.8 Hz, *J* = 8.6 Hz, H_{2''}), 6.89 (d, 1H, *J* = 8.6 Hz, H_{3''}), 6.59 (s, 2H), 5.49 (d, 1H, *J* = 1.1 Hz, H₃), 4.90 (d, 1H, *J* = 1.1 Hz, H₄), 3.92 (s, 3H), 3.78 (s, 3H), 3.72 (s, 6H), 2.20 (s, 3H, OCOCH₃).

¹³C NMR (100 MHz, CDCl₃): δ not determined

Oxazinone 88 was synthesised as a by-product in high yield during the synthesis of 4-(4-methoxy-3-nitrophenyl)-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**25**) using General Method V using phenylacetyl chloride and **54**.



R_f: 0.38 (1:1 *n*-hexane: ethyl acetate)

¹H NMR (600 MHz) CDCl₃: δ 7.71 (d, 1H, *J* = 1.9 Hz, H_{6'''}), 7.42 (dd, 1H, *J* = 2.2 Hz, *J* = 8 Hz, H_{2'''}), 7.34 (t, 1H, *J* = 8.4 Hz, H_{4''}), 7.21 (d, 2H, *J* = 8.3 Hz, H_{2'''}), 7.19 (d, 1H, *J* = 2.1 Hz, H₄), 7.17 (apparent multiplet, 2H, H_{3&5}), 6.93 (apparent m, 2H, H_{2&6}), 6.92 (d, 1H, *J* = 2.8 Hz, H_{3'''}), 6.59 (s, 1H, H₁₁), 6.54 (s, 2H, H_{2'''&6'''}), 3.97 (s, 3H, H_{10'''}), 3.81 (s, 3H, H_{8'''}), 3.8 (s, 6H, H_{7'''&9'''}), 3.53 (d, *J* = 13.8 Hz, 1H, H₇), 3.41 (d, 1H, *J* = 13.8 Hz, H₇)

¹³C NMR (100 MHz, CDCl₃): δ 161.8 (C₁₀), 161.1 (C₈), 153.4 (C_{3'''&5'''}), 153.3 (C_{4'''}), 139.3 (C_{5'''}), 136.8 (C_{4''}), 135.0 (C₁), 134.9 (C_{1'''}), 132.6 (C_{1''}), 132.3 (C_{3'''}), 131.0 (C_{2&6''}), 129.1 (C_{2&6}), 128.8 (C_{1'''}), 128.4 (C_{3&5}), 128.5 (C₃), 128.4 (C₂), 128.4 (C_{3''&5''}), 127.9 (C_{4''}), 127.1 (C₄), 124.6 (C_{3'''}), 115.5 (C₉), 113.0 (C_{6'''}), 103.6 (C_{2'''&6'''}), 88.2 (C₁₁), 60.8 (C_{8'''}), 56.7 (C_{10'''}), 56.3 (C_{7'''&9'''}), 37.5 (C₇)

HRMS: APCI measured for C₃₃H₃₁N₂O₈; 583.27492, found 583.207483, error + 0.0 ppm

Amides **92**, **93**, **95** and **153** were isolated as by-products of **General Method VIII**. They were subsequently isolated using the method detailed below.

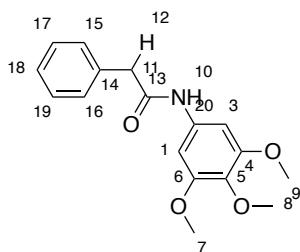
General Method XXIV: Condensation of primary amines and acid chlorides to form novel amide structures *via* base independent dehydration.

3,4,5-trimethoxyaniline (458 mg, 1 eq, 2.5 mmol) was added to a stirring solution of anhydrous toluene (50 mL) with the respective acid chloride (1 eq, 1.5 mmol). The reaction was allowed to stir at room temperature until a white precipitate was formed and starting materials had disappeared as indicated by TLC. The crude reaction mixture was filtered to remove the toluene and the crude product recrystallised from hot ethanol. Selected amides where indicated were isolated spontaneously from the Staudinger reaction crude mixture but not directly synthesised *via* base independent condensation.

2-phenyl-*N*-(3,4,5-trimethoxyphenyl)acetamide (92)

92 was synthesised from phenylacetyl chloride and 3,4,5-trimethoxyaniline using **General Method XXIV**.

It was also isolated during the synthesis of **70** from **47** in the Staudinger reaction.



Yield from General Method XII: 590 mg (1.96 mmol) 78%

R_f: .47 (1:1 *n*-hexane: ethyl acetate)

Appearance: white fluffy crystals

Melting Point: 173-176 °C

¹H NMR (600 MHz, CDCl₃): δ 7.43 (t, 2H, *J* = 8.6 Hz, H_{15&16}), 7.36 (apparent t, 3H, H₁₇₋₁₉), (7.07, bs, 1H, H₁₀), 6.75 (s, 2H, H_{1&3}), 3.82 (s, 6H, H_{7&9}), 3.82 (s, 3H, H₈), 3.75 (s, 2H, H₁₂)

¹³C NMR (100 MHz, CDCl₃): δ 169.0 (C₁₃), 153.3 (C_{4&6}), 134.9 (C₂₀), 134.3 (C₅), 133.7 (C₁₄), 129.7 (C_{17&19}), 129.2 (C_{15&16}), 127.8 (C₁₈), 97.7 (C_{1&3}), 60.9 (C₈), 56.3 (C_{7&9}), 43.1 (C₁₁)

¹⁵N NMR (60.8 MHz, CDCl₃): δ 142.8 (N₁₀)

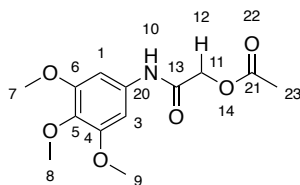
HRMS: APCI measured for C₁₇H₂₀NO₄ [**M**⁺ H⁺]; 302.138685, found 302.138685, error + 0.2 ppm

IR ν_{max}(ATR): 3294 cm⁻¹ (NH)

Purity (RP-HPLC): >95%

2-oxo-2-((3,4,5-trimethoxyphenyl)amino)ethyl acetate (93)

93 was synthesised from acetoxyacetyl chloride and 3,4,5-trimethoxyaniline using **General Method XXIV**. It was also isolated during the synthesis of **17a** and **63a** in the Staudinger reaction.



Yield calculated from Staudinger (17a): 20-30%

Yield from General Method XXIV: 430mg (1.67 mmol) 64%

R_f: 0.21 (1:1 *n*-hexane: ethyl acetate)

Appearance: Grey crystalline powder

Melting Point: 137 °C

¹H NMR (600 MHz, CDCl₃): δ 7.72 (bs, 1H, NH, H₁₀), 6.84 (s, 1H, H_{1&3}), 4.7 (s, 2H, CH₂, H₁₂), 3.88 (s, 6H, H_{7&9}), 3.84 (s, 3H, H₈), 2.26 (s, 3H, H₂₃)

¹³C NMR (100 MHz, CDCl₃): δ 169.3 (C₂₁), 165.0, (C₁₃), 153.4 (C_{4&6}), 135.4 (C₅), 132.7 (C₂₀), 98.0 (C_{1&3}), 63.3 (C₁₁), 61.8 (C₈), 56.2 (C_{7&9}), 20.6 (C₂₃)

¹⁵N NMR (60.8 MHz, CDCl₃): δ 123.5 (N₁₀)

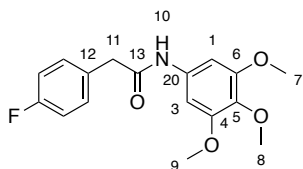
HRMS: APCI measured for C₁₃H₁₆NO₆ [**M**⁻ H⁺]; 282.098311, found 282.097997, error + 1.1 ppm, **ESI** measured for C₁₃H₁₈NO₆ [**M**⁺ H⁺]; 284.112864, found 284.112864, error + 2.1 ppm.

IR ν_{max}(ATR): 3313 cm⁻¹ (NH)

Purity (RP-HPLC): >95%

2-(4-fluorophenyl)-N-(3,4,5-trimethoxyphenyl)acetamide (94)

94 was not isolated from crude Staudinger reaction but synthesised from 4-fluorophenylacetylchloride and 3,4,5-trimethoxyaniline using **General Method XXIV**.



Yield: 422 mg (1.32 mmol) 58%

R_f: 0.42 (1:1 *n*-hexane: ethyl acetate)

Appearance: Grey crystalline powder

Melting Point: 178-179 °C

¹H NMR (400 MHz, CDCl₃): δ 7.32 (apparent m, 2H), 7.11 (apparent triplet, 2H, *J* = 8 Hz), 6.77 (s, 2H, H_{1&3}), 3.84 (s, 6H, H_{7&9}), 3.82 (s, 3H, H₈), 3.72 (s, 2H, H₁₁),

¹³C NMR (100 MHz, CDCl₃): δ 168.9, 163.5, 161.1, 153.4, 135.0, 133.8, 131.1, 130.1, 116.3, 116.0, 97.6, 61.0, 58.5, 56.2, 43.9, 18.5

¹⁹F NMR (376 MHz, CDCl₃): δ -114.3

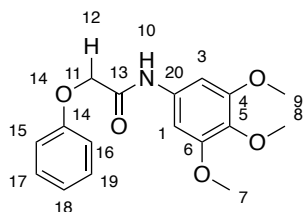
HRMS: APCI measured for C₁₃H₁₆NO₆ [**M** - H⁺]; 318.114710, found 318.114652, error + 0.2 ppm, ESI measured for C₁₇H₁₇FO₄ [**M** - H⁺]; 320.12963, found 320.129138, error -0.4 ppm.

IR ν_{max}(ATR): 3277 cm⁻¹ (NH)

Purity (RP-HPLC): >95%

2-phenoxy-*N*-(3,4,5-trimethoxyphenyl)acetamide (**95**)

95 was synthesised from phenoxyacetyl chloride and 3,4,5-trimethoxyaniline using **General Method XXIV**. It was also isolated during the synthesis of **73** from **47** in the Staudinger reaction.



Yield: 704 mg (2.3 mmol) 89 %

R_f: 0.63 (1:1 *n*-hexane: ethyl acetate)

Appearance: White/silver crystalline powder

Melting Point: 144-145 °C

¹H NMR (400 MHz, CDCl₃): δ 8.26 (bs, 1H, NH, H₁₀), 7.37 (t, 2H, *J* = 7.8 Hz, H_{17&19}), 7.09 (t, 1H, *J* = 5.8 Hz, H₁₈), 7.03 (d, 2H, *J* = 7.8 Hz, H_{15&16}) 6.9 (s, 2H, H_{1&3}), 4.63 (s, 2H, H₁₂), 3.88 (s, 6H, H_{7&9}), 3.84 (s, 3H, H₈)

¹³C NMR (100 MHz, CDCl₃): δ 166.4 (C₁₃), 157.1, 153.5, 135.0, 132.8, 129.9, 122.2, 114.8, 98.1, 67.6, 61.1, 56.2.

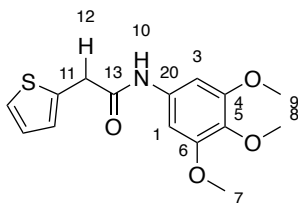
HRMS: APCI measured for C₁₇H₂₀NO₅ [**M** + H⁺]; 318.133599, found 318.133933, error – 1.0 ppm,

IR ν_{max}(ATR): 3287 cm⁻¹ (NH)

Purity (RP-HPLC): >95%

2-(thiophen-2-yl)-*N*-(3,4,5-trimethoxyphenyl)acetamide (**96**)

96 was not isolated from the Staudinger reaction. It was synthesised from 2-thiopheneacetyl chloride and 3,4,5-trimethoxyaniline using **General Method XXIV**.



Yield: 510 mg (1.66 mmol), 66 %

R_f: 0.47 1:1 *n*-hexane: ethyl acetate)

Appearance: pink/grey crystalline powder

Melting Point: 152-153°C

¹H NMR (400 MHz, CDCl₃): δ 7.34 (dd, 1H, *J* = 5.39 Hz, *J* = 1.1 Hz), 7.24 (bs, 1H, NH, H₁₀), 7.07 (m, 3H), 6.75 (s, 2H, H_{1&3}), 3.96 (s, 2H, H₁₂), 3.85 (s, 6H, H_{7&9}), 3.82 (s, 3H, H₈)

¹H NMR (400 MHz, DMSO-*d*₆): δ 10.15 (s, 1H, NH, H₁₀), 7.39 (t, 1H, *J* = 2.3 Hz), 6.98 (apparent s, 4H, H_{1&3} & 3 X thiophene H), 3.8 (s, 2H, H₁₂), 3.74 (s, 6H, H_{7&9}), 3.62 (s, 3H, H₈)

¹³C NMR (100 MHz, DMSO-*d*₆): δ 168.3, 153.2, 137.6, 135.7, 133.8, 127.2, 126.9, 125.6, 97.3, 60.5, 56.1, 38.1

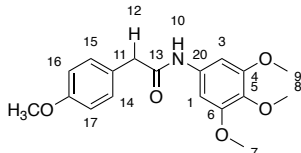
HRMS: APCI measured for C₁₅H₈NO₄S [**M** + **H**⁺]; 308.095105, found 308.095447, error +0.3 ppm,

IR ν_{max}(ATR): 3260 cm⁻¹ (NH)

Purity (RP-HPLC): >95%

2-(4-methoxyphenyl)-*N*-(3,4,5-trimethoxyphenyl)acetamide (**97**)

97 was not isolated from the crude Staudinger reaction. It was synthesised from 4-methoxyphenylacetyl chloride and 3,4,5-trimethoxyaniline using **General Method XXIV**.



Yield: 650 mg (1.95 mmol) 78 %

R_f: 0.37 (1:1 *n*-hexane: ethyl acetate)

Appearance: silver grey crystalline powder

Melting Point: 174-175 °C

¹H NMR (400 MHz, CDCl₃): δ 7.26 (d, 2H, *J* = 7.5 Hz, H_{14&15}), 7.13 (bs, 1H, NH, H₁₀), 6.96 (d, 2H, *J* = 8 Hz, H_{16&17}), 6.75 (s, 2H, H_{1&3}), 3.85 (s, 3H), 3.82 (s, 6H, H_{7&9}), 3.81 (s, 3H), 3.68 (s, 2H, H₁₂).

¹³C NMR (100 MHz, CDCl₃): δ 169.5 (C₁₃), 159.1, 153.5, 134.0, 130.7, 126.1, 115.0, 97.6, 61.0, 56.3, 55.1, 43.4

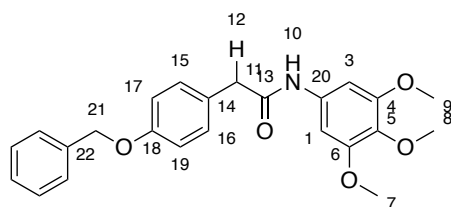
HRMS: APCI measured for C₁₈H₂₀NO₅ [**M** - **H**⁺]; 330.134696, found 330.134547, error - 1.0 ppm,

IR ν_{max}(ATR): 3330 cm⁻¹ (NH)

Purity (RP-HPLC): >95%

2-(4-benzoyloxyphenyl)-*N*-(3,4,5-trimethoxyphenyl)acetamide (**153**)

153 was isolated when synthesising **79a** from **47** and (4-benzoyloxyphenyl)acetic acid. It was not directly synthesised using **General Method XXIV**.



¹H NMR (400 MHz; CDCl₃): δ 7.48-7.33 (m, 5H, Benzyl Ar-H), 7.26 (d, 2H, *J* = 8.1 Hz), 7.06 (bs, 1H, NH, H₁₀), 7.03 (d, 2H, *J* = 8.1 Hz), 6.74 (s, 2H, H_{1&3}), 5.11 (s, 2H, Benzyl CH₂, H₂₁), 3.83 (s, 6H, H_{7&9}), 3.81 (s, 3H, H₈), 3.69 (s, 2H, H₁₂)

¹³C NMR (100 MHz; CDCl₃): δ 169.6 (C₁₃), 158.5, 153.3, 136.5, 130.6, 128.6, 128.1, 127.5, 126.6, 115.7, 97.6, 70.1, 61.0, 56.8, 56.2, 55.9, 47.2, 44.0, 28.3

Numbering nomenclature for 3-hydroxy **EN1** (3*S*,4*S*) and **EN2** (3*R*,4*R*) enantiomers

EN1		EN2	
Enantiomer code		R ₁	R ₂
16EN1		OH	OCH ₃
16EN2			
17EN1		H	OCH ₃
17EN2			
18EN1		F	OCH ₃
18EN2			
19EN1		Cl	OCH ₃
19EN2			
20EN1		CH ₃	OCH ₃
20EN2			
21EN1		Br	OCH ₃
21EN2			
22EN1		H	SCH ₃
22EN2			

(3*S*,4*S*)-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (16EN1)

¹H NMR (400 MHz, CDCl₃): δ 7.29 (s, 1H, OH), 6.65 (apparent s, 1H, H_{6''}), 6.85 (apparent s, 2H, H_{2''} & H_{3''}), 6.53 (s, 2H, H_{1'&3'}), 4.75 (1H, apparent s, H₃), 4.73 (1H, apparent s, H₄), 3.90 (s, 3H, H_{10'}), 3.77 (s, 3H, H_{8'}), 3.71 (s, 6H, (H_{7'&9'}))

¹³C NMR (100 MHz, CDCl₃): δ 167.0 (C¹), 153.8 (C^{4'}&6'), 147.1 (C^{4''}), 147.0 (C^{5''}), 135.0 (C^{5'}), 133.3 (C^{2'}), 130.0 (C^{1''}), 118.2 (C^{2''}), 112.2 (C^{3''}), 111.0 (C^{6''}), 95.5 (C^{1'}&3'), 83.8 (C₄), 65.7 (C₃), 61.0 (C_{8'}), 56.0 (C^{7'}&9'), 56.0 (C_{10'}), 54.0 (C_{8'})

(3R, 4R)-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (16EN2)

¹H NMR (400 MHz, CDCl₃): δ 7.29 (s, 1H, OH), 6.65 (apparent s, 1H, H_{6''}), 6.85 (apparent s, 2H, H_{2''} & H_{3''}), 6.53 (s, 2H, H_{1'}&3'), 4.75 (1H, apparent s, H₃), 4.73 (1H, apparent s, H₄), 3.90 (s, 3H, H_{10'}), 3.77 (s, 3H, H_{8'}), 3.71 (s, 6H, (H_{7'}&9'))

¹³C NMR (100 MHz, CDCl₃): δ 167.0 (C¹), 153.8 (C^{4'}&6'), 147.1 (C^{4''}), 147.0 (C^{5''}), 135.0 (C^{5'}), 133.3 (C^{2'}), 130.0 (C^{1''}), 118.2 (C^{2''}), 112.2 (C^{3''}), 111.0 (C^{6''}), 95.5 (C^{1'}&3'), 83.8 (C₄), 65.7 (C₃), 61.0 (C_{8'}), 56.0 (C^{7'}&9'), 56.0 (C_{10'}), 54.0 (C_{8'})

Dextrorotatory(+)-(3S, 4S)-3-hydroxy-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (17EN1)

¹H NMR (400 MHz, CDCl₃): δ 7.26 Hz (d, 2H, *J*=8.4 Hz, H_{2''}&6''), 6.90 (d, 2H, *J*=8.4 Hz, H_{3''}&5''), 6.5 (s, 2H, H_{1'}&3'), 5.29 (s, 1H, OH), 4.79 (d, 1H, *J*=1.9 Hz, H₄), 4.74 (d, 1H, *J*=1.89 Hz, H₃), 3.80 (s, 3H, H_{10'}), 3.75 (s, 3H, H_{8'}), 3.68 (s, 6H, H_{7'}&9')

¹³C NMR (100 MHz, CDCl₃): δ 167.9 (C₂), 160.0 (C^{4''}), 153.3 (C^{4'}&6'), 135.2 (C^{5'}), 134.2 (C^{2'}), 127.8 (C^{1''}), 127.5 (C^{2''}&6''), 114.5 (C^{3''}&5''), 95.2 (C^{1'}&3'), 83.6 (C₃), 66.0 (C₄), 60.93 (C_{8'}), 55.9 (C^{7'}&9'), 55.3 (C_{10'})

Levorotatory(-)=(3R, 4R)-3-hydroxy-4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (17EN2)

17EN2 was synthesised from **01DS2** using **General Method IX**.

¹H NMR (400 MHz, CDCl₃): δ 7.26 Hz (d, 2H, *J*=8.4 Hz, H_{2''}&6''), 6.90 (d, 2H, *J*=8.4 Hz, H_{3''}&5''), 6.5 (s, 2H, H_{1'}&3'), 5.29 (s, 1H, OH), 4.79 (d, 1H, *J*=1.9 Hz, H₄), 4.74 (d, 1H, *J*=1.89 Hz, H₃), 3.80 (s, 3H, H_{10'}), 3.75 (s, 3H, H_{8'}), 3.68 (s, 6H, H_{7'}&9')

¹³C NMR (100 MHz, CDCl₃): δ 167.9 (C₂), 160.0 (C^{4''}), 153.3 (C^{4'}&6'), 135.2 (C^{5'}), 134.2 (C^{2'}), 127.8 (C^{1''}), 127.5 (C^{2''}&6''), 114.5 (C^{3''}&5''), 95.2 (C^{1'}&3'), 83.6 (C₃), 66.0 (C₄), 60.93= (C_{8'}), 55.9 (C^{7'}&9'), 55.32= (C_{10'})

dextrorotatory-(3S, 4S)-4-(3-fluoro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (18EN1)

¹H NMR (400 MHz, CDCl₃): δ 7.05 Hz (2H, apparent m, H_{2''} & 6''), 6.95 (t, 1H, *J*=8.5 Hz, H_{3''}), 6.47 (s, 2H, H_{7'}&9'), 4.75 (s, 1H, OH), 4.71 (d, 1H, *J*=1.3 Hz, H₄), 4.69 (d, 1H, *J*=1.3 Hz, H₃), 3.88 (s, 3H, H_{10'}), 3.75 (s, 3H, H_{8'}), 3.69 (s, 6H, H_{7'}&9')

¹³C NMR (100 MHz, CDCl₃): δ 166.7 (C₂), 153.8 (C^{4'}&6'), 153.4, 151.4, 148.1, 148.0, 134.8, 132.9, 128.9, 128.8, 122.2, 113.8), 95.3 (C^{1'}&3'), 83.56 (C₄), 65.1 (C₃), 60.9 (C_{8'}), 56.3 (C^{7'}&9'), 56.0 (C_{10'})

¹⁹F NMR (376 MHz, CDCl₃): δ -133.2

levorotatory-(3R, 4R)-4-(3-fluoro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (18EN2)

¹H NMR (400 MHz, CDCl₃): δ 7.05 Hz (2H, apparent m, H_{2''} & 6''), 6.95 (t, 1H, *J*=8.5 Hz, H_{3''}), 6.47 (s, 2H, H_{7'&9'}), 4.75 (s, 1H, OH), 4.71 (d, 1H, *J*=1.3 Hz, H₄), 4.69 (d, 1H, *J*=1.3 Hz, H₃), 3.88 (s, 3H, H_{10'}), 3.75 (s, 3H, H_{8'}), 3.69 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz CDCl₃): δ 166.7 (C₂), 153.8 (C_{4'&6'}), 153.4, 151.4, 148.1, 148.0, 134.8, 132.9, 128.9, 128.8, 122.2, 113.8), 95.3 (C_{1'&3'}), 83.56 (C₄), 65.1 (C₃), 60.9 (C_{8'}), 56.3 (C_{7'&9'}), 56.0 (C_{10'})

¹⁹F NMR (376 MHz, CDCl₃): δ -133.2

(3*S*,4*S*)-4-(3-chloro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (19EN1)

¹H NMR (400 MHz, CDCl₃): δ 7.38 (apparent s, 1H, H_{6''}), 7.21 (d, 1H, *J*=7.5 Hz, H_{2''}), 6.95 (d, 1H, *J*=7.5 Hz, H_{3''}), 6.5 (s, 2H, H_{1'&3'}), 4.77 (apparent s, 1H, H₃), 4.75 (apparent s, 1H, H₄), 3.90 (s, 3H, H_{10'}), 3.78 (s, 3H, H_{8'}), 3.72 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz, CDCl₃): δ 166.7 (C₂), 155.4 (C_{5''}), 153.5 (C_{4'} & C_{6'}), 134.9 (C_{5'}), 132.9 (C_{2'}), 129.1 (C_{1''}), 128.1 (C_{3''}), 125.7 (C_{2''}), 123.4 (C_{1'}), 112.5 (C_{6''}), 95.4 (C_{1'&3'}), 83.1 (C₃), 65.0 (C₄), 61.0 (C_{8'}), 56.3 (C_{10'}), 56.0 (C_{7'&9'})

(3*R*,4*R*)-4-(3-chloro-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (19EN2)

¹H NMR (400 MHz, CDCl₃): δ 7.38 (apparent s, 1H, H_{6''}), 7.21 (d, 1H, *J*=7.5 Hz, H_{2''}), 6.95 (d, 1H, *J*=7.5 Hz, H_{3''}), 6.5 (s, 2H, H_{1'&3'}), 4.77 (apparent s, 1H, H₃), 4.75 (apparent s, 1H, H₄), 3.9- (s, 3H, H_{10'}), 3.78 (s, 3H, H_{8'}), 3.72 (s, 6H, H_{7'&9'}).

¹³C NMR (100 MHz, CDCl₃): δ 166.7 (C₂), 155.4 (C_{5''}), 153.5 (C_{64'} & C_{6'}), 134.9 (C_{5'}), 132.9 (C_{2'}), 129.1 (C_{1''}), 128.1 (C_{3''}), 125.7 (C_{2''}), 123.4 (C_{1'}), 112.5 (C_{6''}), 95.4 (C_{1'&3'}), 83.1 (C₃), 65.0 (C₄), 61.0 (C_{8'}), 56.3 (C_{10'}), 56.0 (C_{7'&9'})

(3*S*,4*S*)-3-hydroxy-4-(4-methoxy-3-methylphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (20EN1)

20EN1 was obtained from **06DS1** using **General Method IX**.

¹H NMR (400 MHz, CDCl₃): δ 7.12 (dd, 1H, *J* = 8.6 Hz, *J* = 1.8 Hz, H_{2''}), 7.10 (apparent s, 1H, H_{6''}), 6.79 (d, 1H, *J* = 8.6 Hz, H_{3''}), 6.52 (s, 2H, H_{1'&3'}), 4.74 (apparent s, 2H, H_{3&4}), 3.82 (s, 3H), 3.76 (s, 3H), 3.69 (s, 6H), 2.20 (s, 3H, B ring *meta* CH₃)

¹H NMR (400 MHz, DMSO-*d*₆): δ 7.14 (dd, 1H, *J* = 8.1 Hz, 2 Hz, H_{2''}), 7.11 (d, 1H, *J* = 1.75 Hz, H_{6''}), 6.81 (d, 1H, *J* = 8.1 Hz, H_{3''}), 6.54 (s, 2H, H_{1'&3'}), 4.77 (d, 1H, *J* = 1.8 Hz, H₄), 4.75 (d, 1H, *J* = 1.8 Hz, H₃), 3.83 (s, 3H, H_{10'}), 3.77 (s, 3H, H_{8'}), 3.71 (s, 6H, H_{7'&9'}), 2.187 (s, 3H, CH₃, H_{5''})

¹³C NMR (100 MHz, DMSO-*d*₆): δ 166.7 (C₂), 158.1 (C_{4''}), 153.4 (C_{4'&6'}), 134.6 (C_{2'}), 133.4 (C_{5'}), 128.4 (C_{3''}), 127.6 (C_{5''}), 127.4 (C_{1''}), 124.9 (C_{2''}), 110.1 (C_{6''}), 95.3 (C_{1'&3'}), 83.6 (C₃), 65.7 (C_{4'}), 60.9 (C_{8'}), 55.9 (C_{7'&9'}), 55.4 (C_{10'}), 30.9 (CH₃)

(3*R*,4*R*)-3-hydroxy-4-(4-methoxy-3-methylphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (20EN2)

¹H NMR (400 MHz, CDCl₃): δ 7.12 (dd, 1H, *J* = 8.6 Hz, *J* = 1.8 Hz, H_{2''}), 7.10 (apparent s, 1H, H_{6''}), 6.79 (d, 1H, *J* = 8.6 Hz, H_{3''}), 6.52 (s, 2H, H_{1'&3'}), 4.74 (apparent s, 2H, H_{3&4}), 3.82 (s, 3H), 3.76 (s, 3H), 3.69 (s, 6H), 2.20 (s, 3H, B ring *meta* CH₃)

¹H NMR (400 MHz, DMSO-*d*₆): δ 7.14 (dd, 1H, *J* = 8.1 Hz, 2 Hz, H_{2''}), 7.11 (d, 1H, *J* = 1.75 Hz, H_{6''}), 6.81 (d, 1H, *J* = 8.1 Hz, H_{3''}), 6.54 (s, 2H, H_{1'&3'}), 4.77 (d, 1H, *J* = 1.8 Hz, H₄), 4.75 (d, 1H, *J* = 1.8 Hz, H₃), 3.83 (s, 3H, H_{10'}), 3.77 (s, 3H, H_{8'}), 3.71 (s, 6H, H_{7'&9'}), 2.187 (s, 3H, CH₃, H_{5''})

¹³C NMR (100 MHz, DMSO-*d*₆): δ 166.7 (C₂), 158.1 (C_{4''}), 153.4 (C_{4'&6'}), 134.6 (C_{2'}), 133.4 (C_{5'}), 128.4 (C_{3''}), 127.6 (C_{5''}), 127.4 (C_{1''}), 124.9 (C_{2''}), 110.1 (C_{6''}), 95.3 (C_{1'&3'}), 83.6 (C₃), 65.7 (C_{4'}), 60.9 (C_{8'}), 55.9 (C_{7'&9'}), 55.4 (C_{10'}), 30.9 (CH₃)

(3*S*,4*S*)-3-hydroxy-4-(4-(methylthio)phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (22EN1)

¹H NMR (400 MHz, CDCl₃): δ 7.23 (apparent s, 3H, H_{6''}, H_{3''}, H_{2''}), 6.5 (s, 2H, H_{1'&3'}), 4.80 (d, 1H, *J* = 1.8 Hz, H₃), 4.73 (apparent s, 1H, H₄), 3.76 (s, 3H, H_{8'}), 3.69 (s, 6H, H_{7'&9'}), 2.48 (s, SCH₃, H_{10'})

¹H NMR (400 MHz, DMSO-*d*₆): δ 7.38 (d, 1H, H_{2''&6''}), 7.28 (d, 2H, *J* = 8.6 Hz, H_{3''&5''}), 6.72 (d, 1H, *J* = 8.6 Hz, 3-OH), 6.52 (s, 2H, H_{1'&3'}), 4.89 (d, 1H, *J* = 1.9 Hz, H₄), 4.75 (dd, 1H, *J* = 7.6 Hz, *J* = 1.9 Hz, H₃), 3.63 (s, 6H, H_{7&9}), 3.57 (s, 3H, H_{8'}), 2.46, (s, 3H, H_{10'}).

¹³C NMR (100 MHz, DMSO-*d*₆): δ 166.9, 153.5, 138.9, 134.3, 133.8, 133.4, 127.9, 126.6, 83.8, 65.1, 60.5, 56.3, 14.9

(3*R*,4*R*)-3-hydroxy-4-(4-(methylthio)phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (22EN2)

¹H NMR (400 MHz, CDCl₃): δ 7.23 (apparent s, 3H, H_{6''}, H_{3''}, H_{2''}), 6.5 (s, 2H, H_{1'&3'}), 4.80 (d, 1H, *J* = 1.8 Hz, H₃), 4.73 (apparent s, 1H, H₄), 3.76 (s, 3H, H_{8'}), 3.69 (s, 6H, H_{7'&9'}), 2.48 (s, SCH₃, H_{10'})

¹H NMR (400 MHz, DMSO-*d*₆): δ 7.38 (d, 1H, H_{2''&6''}), 7.28 (d, 2H, *J* = 8.6 Hz, H_{3''&5''}), 6.72 (d, 1H, *J* = 8.6 Hz, 3-OH), 6.52 (s, 2H, H_{1'&3'}), 4.89 (d, 1H, *J* = 1.9 Hz, H₄), 4.75 (dd, 1H, *J* = 7.6 Hz, *J* = 1.9 Hz, H₃), 3.63 (s, 6H, H_{7&9}), 3.57 (s, 3H, H_{8'}), 2.46, (s, 3H, H_{10'}).

¹³C NMR (100 MHz, DMSO-*d*₆): δ 166.9, 153.5, 138.9, 134.3, 133.8, 133.4, 127.9, 126.6, 83.8, 65.1, 60.5, 56.3, 14.9

(3*S*,4*S*)-4-(3-bromo-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (21EN1)

¹H NMR (400 MHz, CDCl₃): δ 7.57 (d, 1H, *J* = 1.6 Hz, H_{6''}), 7.25 (dd, 1H, *J* = 8.3 Hz, *J* = 1.8 Hz, H_{2''}), 6.91 (d, 1H, *J* = 8.3 Hz, H_{3''}), 6.51 (s, 1H, H_{1'&3'}), 4.78 (d, 1H, *J* = 1.5 Hz, H₃), 4.74 (d, 1H, *J* = 1.5 Hz, H₄), 3.92 (s, 3H, H_{10'}), 3.78 (s, 3H, H_{8'}), 3.73 (s, 6H, H_{7'&9'})

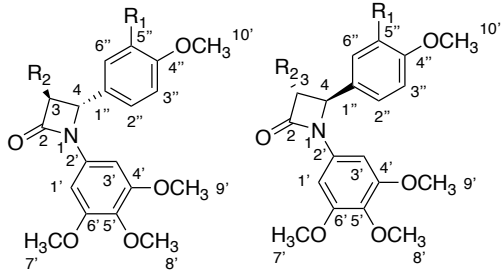
¹³C NMR (100 MHz, CDCl₃): δ 165.9 (C₂), 157.2 (C_{4''}), 153.4 (C_{4'&6'}), 134.9 (C_{5'}), 132.9 (C_{2'}), 131.2 (C_{6''}), 129.5 (C_{1''}), 126.3 (C_{2''}), 112.5 (C_{5''}), 112.3 (C_{3''}), 95.4 (C_{1'&3'}), 86.6 (C₃), 64.4 (C₄), 60.9 (C_{8'}), 56.4 (C_{7'&9'}), 56.1 (C_{10'})

(3*R*,4*R*)-4-(3-bromo-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (21EN2)

¹H NMR (400 MHz, CDCl₃): δ 7.57 (d, 1H, *J* = 1.6 Hz, H_{6''}), 7.25 (dd, 1H, *J* = 8.3 Hz, *J* = 1.8 Hz, H_{2''}), 6.91 (d, 1H, *J* = 8.3 Hz, H_{3''}), 6.51 (s, 1H, H_{1'&3'}), 4.78 (d, 1H, *J* = 1.5 Hz, H₃), 4.74 (d, 1H, *J* = 1.5 Hz, H₄), 3.92 (s, 3H, H_{10'}), 3.78 (s, 3H, H_{8'}), 3.73 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz, CDCl₃): δ 165.9 (C₂), 157.2 (C_{4''}), 153.4 (C_{4'&6'}), 134.9 (C_{5'}), 132.9 (C_{2'}), 131.2 (C_{6''}), 129.5 (C_{1''}), 126.3 (C_{2''}), 112.5 (C_{5''}), 112.3 (C_{3''}), 95.4 (C_{1'&3'}), 86.6 (C₃), 64.4 (C₄), 60.9 (C_{8'}), 56.4 (C_{7'&9'}), 56.1 (C_{10'})

Numbering nomenclature for 3-substituted enantiomers



Enantiomer Code	R ₁	R ₂
27EN1	H	OH
27EN2	OH	H
30EN1	OH	
30EN2	H	
33EN1	OH	

4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (27EN1)

(absolute configuration not determined)

¹H NMR (400 MHz, CDCl₃): δ 6.98 (d, 1H, ³J_{6''-2''} = 1.8 Hz, H_{6''}), 6.91 (dd, 1H, ³J_{2''-3''} = 8.4 Hz, ³J_{2''-6''} = 1.3 Hz, H_{2''}), 6.86 (d, 1H, ³J_{3''-2''} = 8.3 Hz, H_{3''}), 6.58 (s, 2H, H_{1'&3'}), 5.7 (bs, 1H, H₈, OH), 4.89 (dd, 1H, ³J₄₋₅ = 5.5 Hz, ³J₄₋₃ = 2.7 Hz, H₄), 3.92 (s, 3H, H_{10'}), 3.78 (s, 3H, H_{8'}), 3.75 (s, 6H, H_{7'&9'}), 3.53 (dd, 1H, ²J₅₋₃ = 13.7 Hz, ³J₅₋₄ = 5.1 Hz, H₅), 2.95 (dd, 1H, ³J₃₋₅ = 14.6 Hz, ³J₃₋₅ = 2.7 Hz, H₃)

¹³C NMR (100 MHz, CDCl₃): δ 165.0, 153.5, 146.1, 117.7, 112.0, 110.9, 94.6, 77.3, 76.7, 60.9, 56.1, 54.1, 46.9

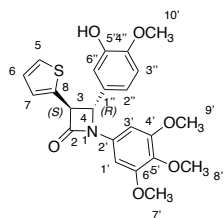
4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (27EN2)

(absolute configuration not determined)

¹H NMR (400 MHz, CDCl₃): δ 6.98 (d, 1H, ³J_{6''-2''} = 1.8 Hz, H_{6''}), 6.91 (dd, 1H, ³J_{2''-3''} = 8.4 Hz, ³J_{2''-6''} = 1.3 Hz, H_{2''}), 6.86 (d, 1H, ³J_{3''-2''} = 8.3 Hz, H_{3''}), 6.58 (s, 2H, H_{1'&3'}), 5.7 (bs, 1H, H₈, OH), 4.89 (dd, 1H, ³J₄₋₅ = 5.5 Hz, ³J₄₋₃ = 2.7 Hz, H₄), 3.92 (s, 3H, H_{10'}), 3.78 (s, 3H, H_{8'}), 3.75 (s, 6H, H_{7'&9'}), 3.53 (dd, 1H, ²J₅₋₃ = 13.7 Hz, ³J₅₋₄ = 5.1 Hz, H₅), 2.95 (dd, 1H, ³J₃₋₅ = 14.6 Hz, ³J₃₋₅ = 2.7 Hz, H₃)

¹³C NMR (100 MHz, CDCl₃): δ 165.0, 153.5, 146.1, 117.7, 112.0, 110.9, 94.6, 77.3, 76.7, 60.9, 56.1, 54.1, 46.9

(3S, 4R)-4-(3-hydroxy-4-methoxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (30EN1)

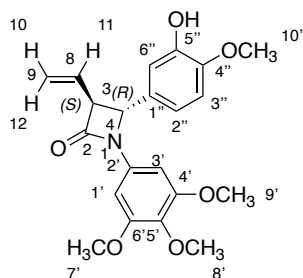


¹H NMR (400 MHz, CDCl₃): δ 7.31 (dd, 1H, *J* = 7 Hz, *J* = 1.7 Hz), 7.10 (d, 1H, *J* = 1.8 Hz), 7.05 (dd, 1H, *J* = 8.41 Hz, *J* = 3.5 Hz, H₆), 7.01 (d, 1H, *J* = 2.1 Hz), 6.94 (dd, 1H, *J* = 8.14 Hz, *J* = 1.9 Hz), 6.89 (d, 1H, *J* = 8.9 Hz, H_{3''}), 6.61 (s, 2H, H_{1'&3'}), 5.70 (bs, 1H, OH), 4.87 (d, 1H, *J* = 1.8 Hz, H₄), 4.48 (d, 1H, *J* = 1.8 Hz, H₃), 3.93 (s, 3H, H_{10'}), 3.80 (s, 3H, H_{8'}), 3.76 (s, 6H, H_{7'&9'})
¹³C NMR (100 MHz, CDCl₃): δ 164.4 (C₂), 153.6 (C_{4'&6'}), 146.9 (C_{4''}), 146.4 (C_{5''}), 136.1, 134.7, 133.6, 129.9, 127.3, 125.8, 125.3, 117.7, 112.0, 111.0, 94.9, 64.5, 60.9, 60.1, 56.05

(3R,4S)-4-(3-hydroxy-4-methoxyphenyl)-3-(thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (30EN2)

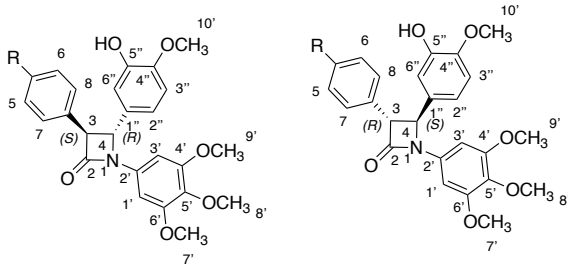
¹H NMR (400 MHz, CDCl₃): δ 7.31 (dd, 1H, *J* = 7 Hz, *J* = 1.7 Hz), 7.10 (d, 1H, *J* = 1.8 Hz), 7.05 (dd, 1H, *J* = 8.41 Hz, *J* = 3.5 Hz, H₆), 7.01 (d, 1H, *J* = 2.1 Hz), 6.94 (dd, 1H, *J* = 8.14 Hz, *J* = 1.9 Hz), 6.89 (d, 1H, *J* = 8.9 Hz, H_{3''}), 6.61 (s, 2H, H_{1'&3'}), 5.70 (bs, 1H, OH), 4.87 (d, 1H, *J* = 1.8 Hz, H₄), 4.48 (d, 1H, *J* = 1.8 Hz, H₃), 3.93 (s, 3H, H_{10'}), 3.80 (s, 3H, H_{8'}), 3.76 (s, 6H, H_{7'&9'})
¹³C NMR (100 MHz, CDCl₃): δ 164.4 (C₂), 153.6 (C_{4'&6'}), 146.9 (C_{4''}), 146.4 (C_{5''}), 136.1, 134.7, 133.6, 129.9, 127.3, 125.8, 125.3, 117.7, 112.0, 111.0, 94.9, 64.5, 60.9, 60.1, 56.05

(3S,4R)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-3-vinylazetidin-2-one (33EN1)



¹H NMR (400 MHz CDCl₃): δ 6.97 (d, 1H, H_{6''}, ³*J*_{6''-2''} = 2.1 Hz), 6.88 (dd, 1H, ³*J*_{2''-3''} = 8 Hz, *J*_{2''-6''} = 2.1 Hz, H_{2''}), 6.87 (d, 1H ³*J*_{3''-2''} = 8 Hz, H_{3''}), 6.58 (s, 2H, H_{1'&3'}), 6.03 (td, 1H, *J*₁₁₋₁₂ = 17.1 Hz, *J*₁₁₋₁₀ = 9.9 Hz, *J*₁₁₋₃ = 2.2 Hz, H₁₁), 5.71 (bs, OH), 5.40 (dd, 1H, ³*J*₁₂₋₁₁ = 17 Hz, ²*J*₁₂₋₁₀ (geminal) = 2.2 Hz, H₁₂), 5.33 (dd, 1H, ³*J*₁₀₋₁₁ = 9.9 Hz, ²*J*₁₀₋₁₂ (geminal) = 2.2 Hz, H₁₀), 4.69 (d, 1H, *J*₄₋₃ = 2.2 Hz, H₄), 3.92 (s, 3H, H_{8'}), 3.78 (s, 3H, H_{10'}), 3.75 (s, 6H, H_{7'&9'}), 3.74 (dt, 1H, ³*J*₃₋₁₁ = 8.5 Hz, ³*J*₃₋₄ = 2.2 Hz, H₃)
¹³C NMR (100 MHz CDCl₃): δ 165.3 (C₂), 153.5 (C_{1'&3'}), 146.9 (C_{4''}), 146.3 (C_{5''}), 135.2 (C_{5'}), 133.9 (C_{2'}), 130.6 (C_{1''}), 130.5 (C₉), 119.8 (C₈), 117.8 (C_{2''}), 112.0 (C_{6''}), 111.9 (C_{3''}), 94.8 (C_{1'&3'}), 63.9 (C₃), 61.4 (C₄), 60.9 (C_{8'}), 56.1 (C_{7'&9'}), 56.0 (C_{10'})

Numbering nomenclature for 3-phenyl-substituted 3*S*,4*R* **EN1** and 3*R*,4*S* **EN2** enantiomers

	
EN1	EN2
Enantiomer code	R
24EN1	H
24EN2	
39EN1	F
39EN2	
41EN1	OCH ₃
41EN2	

(3*S*, 4*R*)-4-(3-Hydroxy-4-methoxyphenyl)-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (24EN1)

¹H NMR (400 MHz, CDCl₃): δ 7.35 (m, 5H, 3-phenyl, H_{5,8}), 6.99 (d, 1H, *J*=2 Hz, H_{6''}), 6.91 (dd, 1H, H_{2'}, *J*=8 Hz, *J*=2 Hz, H_{2''}), 6.86 (d, 1H, *J*=8 Hz, H_{3''}), 6.64 (s, 2H, H_{1'&3'}), 5.79 (s, OH), 4.80 (d, 1H, *J*=2.5 Hz, H₄), 4.29 (d, 1H, *J*=2.5 Hz, H₃), 3.90 (s, 3H, H_{10'}), 3.76 (s, 3H, H_{8'}), 3.72 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz, CDCl₃): δ 165.5 (C₂), 153.4 (C_{4'&6'}), 146.8 (C_{4''}), 146.3 (C_{5''}), 134.7 (C_{5'}), 133.7 (C_{2'}), 129.0 (C_{1''}), 127.87, 127.4, 117.7 (C_{2''}), 112.0 (C_{3''}), 110.9 (C_{6''}), 94.9 (C_{1'&3'}), 64.92 (C₃), 63.7 (C₄), 61.0 (C_{8'}), 56.0 (C_{7'&9'}), 55.9 (C_{10'})

(3*R*, 4*S*)-4-(3-Hydroxy-4-methoxyphenyl)-3-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (24EN2)

¹H NMR (400 MHz, CDCl₃): δ 7.35 (m, 5H, 3-phenyl, H_{5,8}), 6.99 (d, 1H, *J*=2 Hz, H_{6''}), 6.91 (dd, 1H, H_{2'}, *J*=8 Hz, *J*=2 Hz, H_{2''}), 6.86 (d, 1H, *J*=8 Hz, H_{3''}), 6.64 (s, 2H, H_{1'&3'}), 5.79 (s, OH), 4.80 (d, 1H, *J*=2.5 Hz, H₄), 4.29 (d, 1H, *J*=2.5 Hz, H₃), 3.90 (s, 3H, H_{10'}), 3.76 (s, 3H, H_{8'}), 3.72 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz, CDCl₃): δ 165.5 (C₂), 153.4 (C_{4'&6'}), 146.8 (C_{4''}), 146.3 (C_{5''}), 134.7 (C_{5'}), 133.7 (C_{2'}), 129.0 (C_{1''}), 127.87, 127.4, 117.7 (C_{2''}), 112.0 (C_{3''}), 110.9 (C_{6''}), 94.9 (C_{1'&3'}), 64.92 (C₃), 63.7 (C₄), 61.0 (C_{8'}), 56.0 (C_{7'&9'}), 55.9 (C_{10'})

(3*S*, 4*R*)-3-(4-fluorophenyl)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (39EN1)

¹H NMR (400 MHz, CDCl₃): δ 7.33 (apparent dd, 2H, *J*=8.1 Hz, *J*=3.1 Hz, H_{5&6}), 7.09 (apparent triplet, 2H, *J*=8.1 Hz, H_{7&8}), 7.00 (d, 1H, *J*=2.1 Hz, H_{6''}), 6.93 (dd, 1H, *J*=7.9, *J*=1.8 Hz, H_{2''}), 6.89 (d, 1H, *J*=7.9, H_{3''}), 6.63 (s, 2H, H_{1'&3'}), 5.72 (s, OH), 4.78 (d, 1H, *J*=1.8 Hz, H₃), 4.27 (d, 1H, *J*=1.8 Hz, H₄), 3.94 (s, 3H, H_{10'}), 3.80 (s, 3H, H_{8'}), 3.76 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz, CDCl₃): δ 161.3 (C₂), 153.6 (C_{4'&6'}), 147.0, 146.4, 130.3, 129.0, 117.8, 116.2, 115.9, 115.3, 112.0, 111.2, 94.9 (C_{1'&3'}), 85.05 (C₃), 64.3 (C₄), 64.0, 64.0, 61.1, 56.2 (C_{7'&9'})

¹⁹F NMR (376 MHz, CDCl₃): δ -114.1

(3R,4S)-3-(4-fluorophenyl)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (39EN2)

¹H NMR (400 MHz, CDCl₃): δ 7.33 (apparent dd, 2H, *J* = 8.1 Hz, *J* = 3.1 Hz, H_{5&6}), 7.09 (apparent triplet, 2H, *J* = 8.1 Hz, H_{7&8}), 7.00 (d, 1H, *J* = 2.1 Hz, H_{6''}), 6.93 (dd, 1H, *J* = 7.9, *J* = 1.8 Hz, H_{2''}), 6.89 (d, 1H, *J* = 7.9, H_{3''}), 6.63 (s, 2H, H_{1'&3'}), 5.72 (s, OH), 4.78 (d, 1H, *J* = 1.8 Hz, H₃), 4.27 (d, 1H, *J* = 1.8 Hz, H₄), 3.94 (s, 3H, H_{10'}), 3.80 (s, 3H, H_{8'}), 3.76 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz CDCl₃): δ 161.3 (C₂), 153.6 (C_{4'&6'}), 147.0, 146.4, 130.3, 129.0, 117.8, 116.2, 115.9, 115.3, 112.0, 111.2, 94.9 (C_{1'&3'}), 85.05 (C₃), 64.3 (C₄), 64.0, 64.0, 61.1, 56.2 (C_{7'&9'})

¹⁹F NMR (376 MHz, CDCl₃): δ -114.1

(3S,4R)-4-(3-hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (41EN1)

¹H NMR (400 MHz CDCl₃): δ 7.25 (d, 2H, *J* = 8.2 Hz, H_{7&8}), 7.0 (d, 1H *J* = 2 Hz, H_{6''}), 6.92 (d, 2H, *J* = 8.2 Hz, H_{5&56}), 6.92 (dd, *J* = 8.1 Hz, *J* = 2 Hz, H_{2''}), 6.9 (d, *J*_{3''-2''} = 8.1 Hz, H_{3''}), 6.63 (s, 2H, H_{1'&3'}), 5.75 (broad s, OH), 4.77 (d, 1H, *J* = 2.1 Hz, H₃), 4.23 (d, 1H, *J* = 2.1 Hz, H₄), 3.92 (s, 3H, H_{10'}), 3.83 (s, 3H, H_{7''}), 3.79 (s, 3H, H_{8'}), 3.75 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz CDCl₃): δ 166.0 (C₂), 159.3 (C_{4'''}), 153.5 (C_{4'&6'}), 146.8, 146.4, 134.5 (C_{5'}), 133.8 (C_{2'}), 130.7, 128.6 (C_{6''}), 126.9 (C_{2''}), 117.8 (C_{6''}), 114.4, 112.1 (C_{3''}), 111.0, 94.9 (C_{1&3}), 64.5 (C₄), 64.2 (C₃), 56.1 (C_{7'&9'}), 56.0 (C_{10'}), 55.3 (C_{7'''})

(3R,4S)-4-(3-hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (41EN2)

¹H NMR (400 MHz CDCl₃): δ 7.25 (d, 2H, *J* = 8.2 Hz, H_{7&8}), 7.0 (d, 1H *J* = 2 Hz, H_{6''}), 6.92 (d, 2H, *J* = 8.2 Hz, H_{5&56}), 6.92 (dd, *J* = 8.1 Hz, *J* = 2 Hz, H_{2''}), 6.9 (d, *J*_{3''-2''} = 8.1 Hz, H_{3''}), 6.63 (s, 2H, H_{1'&3'}), 5.75 (broad s, OH), 4.77 (d, 1H, *J* = 2.1 Hz, H₃), 4.23 (d, 1H, *J* = 2.1 Hz, H₄), 3.92 (s, 3H, H_{10'}), 3.83 (s, 3H, H_{7''}), 3.79 (s, 3H, H_{8'}), 3.75 (s, 6H, H_{7'&9'})

¹³C NMR (100 MHz CDCl₃): δ 166.0 (C₂), 159.3 (C_{4'''}), 153.5 (C_{4'&6'}), 146.8, 146.4, 134.5 (C_{5'}), 133.8 (C_{2'}), 130.7, 128.6 (C_{6''}), 126.9 (C_{2''}), 117.8 (C_{6''}), 114.4, 112.1 (C_{3''}), 111.0, 94.9 (C_{1&3}), 64.5 (C₄), 64.2 (C₃), 56.1 (C_{7'&9'}), 56.0 (C_{10'}), 55.3 (C_{7'''})

General Method XXV: Addition of dimethylphosphite to P3 to afford P3

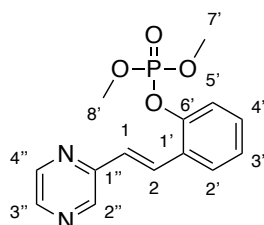
dimethylphosphate

P3 (250mg, 1.16 mmol, 1 eq) was added to a stirring solution of anhydrous MeCN (20 mL) under a nitrogen atmosphere and sonicated for 10 minutes. The reaction vessel was then placed on ice followed by dropwise addition of CCl₄ (0.4 mL, 3.5 mmol, 3 eq). The solution was stirred vigorously for 20 minutes. DMAP (16mg, 0.125 mmol, 0.1 eq) and DIPEA (0.45 mL, 2.52 mmol, 2 Eq) were dissolved in MeCN (5 mL) and injected. The solution was stirred for a further 5 minutes before the addition of dimethylphosphite (0.16 mL, 1.8 mmol, 1.4 eq). The solution was stirred for a further 4 hours and monitored by TLC until a decrease in R_f was observed. The reaction was then quenched with KHSO_{4(sat)} (20mL). The reaction was diluted with ethyl acetate (20 mL). After phase separation the aqueous layer was retained and was then

extracted with 3 X 20 mL ethyl acetate. The combined organic layers were washed with brine and dried using anhydrous Na₂SO₄. A crude yellow oil was obtained. The purified product was isolated from parent material using flash chromatography over silica gel with a gradient of ethyl acetate: MeOH (100:0 - 80:20).

(E)-dimethyl (2-(2-(pyrazin-2-yl)vinyl)phenyl) phosphate (P3DMP)

P3DMP was synthesised from **P3** and dimethyl phosphite using **General Method XXV**.



Yield: 160mg, 50%

Appearance: dark brown oil

R_f: 2:1 *n*-hexane: ethyl acetate: 0.16 (plate development 1), 0.22 (plate development 2)

¹H NMR (600 MHz, DMSO-*d*₆): δ 8.76 (d, 1H, *J*_{4''-3''} = 1.6 Hz, H_{4''}), 8.67 (t, 1H, *J*_{3''-2''} = 2.3 Hz, *J*_{3''-4''} = 1.6 Hz, H_{3''}), 8.53 (d, 1H, *J*_{2''-3''} = 2.2 Hz, H_{2''}), 8.01 (d, *J*₂₋₁ = 16 Hz, H₂), 7.93 (d, 7.47 *J*_{2'-3'} = 7.67 Hz, H_{2'}) (d, *J*₁₋₂ = 16 Hz, H₁), 7.43 (dt, *J*_{P=4'} = 1.6 Hz, *J*_{4'-5'-3'} = 8.1 Hz, H_{4'}), 7.32 (apparent multiplet, 2H, H_{3'&5'}), 3.84 (s, 3H, P-O-CH₃), 3.83 (s, 3H, P-O-CH₃)

¹³C NMR (100 MHz, DMSO-*d*₆): δ 150.7 (C_{1''}), 148.6 (C_{6'}), 145.2 (C_{3''}), 144.7 (C_{4''}), 144.1 (C_{2''}), 130.7 (C_{1'}), 128.0 (C_{2'}), 127.7 (C₂), 126.9 (C₁), 126.1 (C_{3'}), 120.9 (C_{5'}), 55.5 (C_{7'&8'})

³¹P NMR (162 MHz, DMSO-*d*₆): δ -3.6

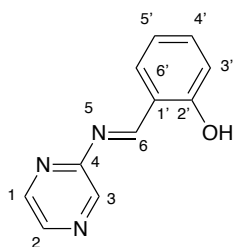
HRMS: APCI Calculated for C₁₄H₁₆N₂O₄P [**M-H**⁺]: 307.084220, found 307.084214, error + 0 ppm. APCI calculated for C₁₄H₁₅N₂NaO₄P [**M+Na**⁺]: 329.06611, found 329.066428, error +0.8 ppm

General Method XXVI: Synthesis of P3 phosphoric acid using phosphorous oxychloride

Pyrazinib (50mg, 0.25mmol, 1 eq) was added to a stirring solution of anhydrous THF (30 mL) and sonicated for 20 minutes under a nitrogen atmosphere, prior to commencing the reaction to aid dissolution of the solids. The reaction vessel was then cooled on an ice/NaCl bath to -15 °C and set up under reflux conditions to allow for condensation of THF following addition of reagents. Phosphorous oxychloride (0.03 mL, 0.3 mmol, 1.2 eq) was added dropwise to the vessel *via* injection. Following cooling of the vessel, anhydrous TEA was added dropwise (0.04 mL, 1.2 mmol, 1.2 eq). The reaction vessel was allowed to warm to room temperature and stirred for 3 hours. dH₂O (20 mL) was added and stirred overnight. The solvents were reduced *in vacuo* and the residue dissolved in methanol (30mL) before drying with anhydrous Na₂SO₄. The solvent was once again removed in *vacuo* to afford the crude phosphoric acid intermediate in complex with TEA.

2-((pyrazin-2-ylimino)methyl)phenol (146)

146 was synthesised from 2-aminopyrazine and salicylaldehyde using **General Method III**.



Yield: >90% **Appearance:** yellow crystalline material

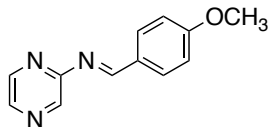
¹H NMR (600 MHz); CDCl₃: δ 13.00 (bs, OH), 9.48 (s, 1H, H₆), 8.707 (d, 1H, H₃, *J*=1.6Hz), 8.53 (d, 1H, *J*= 1.9Hz, H₁), 8.51(dd, 2H, *J*=16Hz, *J*= 2.5Hz, H₂), 7.54 (d, 1H, *J*= 8.5Hz, H_{6'}), 7.48(t, 1H, *J*= 8Hz, *J*= 2Hz, H_{5'}), 7.083 (d, 1H, *J*= 8.2Hz, *J*= 2Hz, H_{3'}), 7.01(t, 1H, *J*= 8Hz, *J*= 2Hz, H_{4'})

¹³C NMR (100 MHz); CDCl₃: δ 166.9 (C₆), 162.1 (C_{1'}), 153.8 (C₃), 143.2 (C₂), 143.4 (C₂), 143.2 (C₂), 142.6(C₃), 134.8 (C_{5'}), 133.9 (C_{6'}), 117.5 (C_{3'})

HRMS: APCI calculated for C₁₁H₉N₃O [**M⁺ +H**], 200.081612; found 200.081838

(*E*)-1-(4-methoxyphenyl)-*N*-(pyrazin-2-yl)methanimine (150)

150 was synthesised from 2-aminopyrazine and 4-methoxybenzaldehyde (*para*-anisaldehyde) using **General Method XXI** and **General Method XXII**.



HRMS: APCI calculated for C₁₂H₁₂N₃O [**M⁺ +H**], 214.097488; found 214.097617

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